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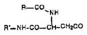
(54) Title: SYNTHETIC LIPID-A ANALOGS AND USES THEREOF



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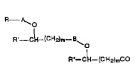

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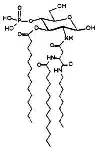

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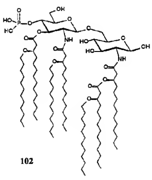

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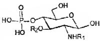

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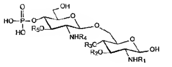

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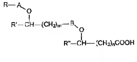

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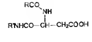

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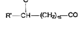

(III)

(57) Abstract: New synthetic Lipid-A analogs based on monosaccharide (1) and disaccharide (2) derivatives were designed and prepared in the present invention. Both structures (1) and (2) incorporate novel lipid structures (3) and (4) that are not found in nature. Also, novel disaccharide Lipid-A structures (2) that incorporate novel contingents of uniform lipids and where R1, R4 and R5 are the same substitution group of structure (III) were synthesized. Liposome formulations containing totally synthetic components such as synthetic Lipid-A and synthetic lipopeptide derived from tumor-associated MUC1 mucin are described along with their therapeutic utility. Comparative test results of immunostimulating properties and toxicity of Lipid-A analogs (1) and (2) are included.

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Synthetic Lipid-A Analogs and Uses Thereof

This application is a non-provisional application of the provisional US application serial No. 60/164,928 filed on Nov. 15, 1999. This prior provisional application is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to novel synthetic structural mimics of bacterial Lipid-A and methods of synthesis of such analogs. Bacterial Lipid-A compositions are being widely used as adjuvant to enhance the immune responses to various antigens used in vaccine formulations. A synthetic adjuvant, being a single chemically defined entity, leads to the required homogeneity for vaccine formulations of either liposomal origin or normal admixtures. This invention includes the design and synthesis of Lipid-A analogs, with much lower toxicity but with adjuvant properties comparable to those of the natural Lipid-A. These synthetic structures incorporate unnatural lipids, unnatural chemical linkages and combinations of lipid chains that are not found among natural lipid-A structures.

BACKGROUND OF THE INVENTION

Lipopolysaccharide (LPS) is a unique glycolipid found exclusively in the outer leaflet of the outer membrane of Gram-negative bacteria. Structurally[1a, b], bacterial LPS molecule has three main regions: the O-antigen region, the core region and the Lipid-A region. The O-antigen region is a strain-specific polysaccharide moiety and determines the antigenic specificity of the organism. The core region is an oligosaccharide chain and may play a role in maintaining the integrity of the outer membrane. The Lipid-A region is conserved and functions as a hydrophobic anchor holding lipopolysaccharide in place.

LPS is known to trigger many pathophysiological events in mammals, either when it is injected or when is accumulated due to Gram-negative bacterial infection[1b]. Before the discovery of

Lipid-A component of LPS the term "endotoxin" was generally used to describe the effects of the LPS. The endotoxin from Gram-negative bacteria is heat-stable, cell associated, pyrogenic and potentially lethal. In addition to its endotoxic activities, LPS also exhibits various biological activities, which include immuno adjuvant activity, B-lymphocyte mitogenesis, macrophage activation, interferon production, tumor regression, etc. While both the O-antigen and the core regions modulate the toxic activity of the LPS, it is generally believed that the hydrophobic Lipid-A moiety is responsible for these pathophysiological effects of the endotoxin[2a, b].

Lipid-A consists of a β-(1,6)-linked D-glucosamine disaccharide phosphorylated at 1-O- and 4'-O-positions. Hydroxylated and non-hydroxylated fatty acids are linked to the hydroxyl and amino groups of the disaccharide to confer hydrophobicity to the Lipid-A. FIG. 1 shows two examples of natural Lipid-A structures, compound A[3a, b] isolated from *E. coli*, and compound B[4a-d] isolated from *Salmonella* strains. Takada and Kotani have conducted a thorough study of structural requirements of Lipid-A for endo-toxicity and other biological activities[5a], thanks to the availability of synthetic Lipid-A analogs due to the efforts of various groups[6-10]. Ribi et al[5b] showed that the minimal structure required for toxicity was a bisphosphorylated β-(1,6)-linked di-glucosamine core to which long chain fatty acids are attached. It appears that an optimal number of lipid chains, in the form of either hydroxy acyl or acyloxyacyl groups, are required on the disaccharide backbone in order to exert strong endotoxic and related biological activities of Lipid-A[6]. For immunoadjuvant activity, however, the structural requirements of Lipid-A do not appear to be as rigid as those required for endotoxic activity and IFN-α/β or TNF-inducing properties[5]. Removal of all fatty acids, however, abrogates all biological activities normally attributed to Lipid-A.

In addition, removal of either phosphate group results in significant loss of toxicity without a corresponding loss of adjuvant activity. Bioassays on monophosphoryl Lipid-A showed that, while it was 1000 times less potent on a molar basis in eliciting toxic and pyrogenic responses, it was comparable to diphosphoryl Lipid-A (and endotoxin itself) in immunostimulating activities[11a]. It is known that the diphosphoryl Lipid-A from *E. coli* and *Salmonella* strains are highly toxic, but the monophosphoryl Lipid-A from *E. coli* has reduced toxicity while retaining the numerous biological activities that are normally associated with LPS[11 b, c, d].

The potent biological activities of Lipid-A have directed numerous research efforts toward developing useful applications. For example, the inhibition of Lipid-A biosynthesis is a new target activity for antibacterial drugs[12, 13], and the drugs of future that function through this inhibitory mechanism will constitute a new class of antibiotics[14, 15]. The immunostimulating activity of Lipid-A has been investigated in order to develop new therapeutic anti-tumor agents[16, 17] and immunoadjuvants by using modified Lipid-A structures and analogs. Furthermore, therapeutic agents of Lipid-A analogs have been investigated for treatment of sepsis[18] based on their abilities to inhibit the interaction with macrophages, and as antagonists for the toxic activity of Lipid-A. Recently, Eisai[19] developed a potent synthetic Lipid-A antagonist for the treatment of sepsis.

There is a need for effective treatment for Lipid-A / LPS associated disorders, and for a potent adjuvant without the associated toxicity. The high toxicity of unmodified Lipid-A from natural source prevents its general use as a pharmaceutical. A major drawback with the naturally derived Lipid-A is in accessing sufficient material with pharmaceutically acceptable purity, reproducible activity and stability. Naturally derived Lipid-A is a mixture of several components of cell wall including those of Lipid-A with varying number of lipid chains. Such heterogeneity in natural Lipid-A product is attributed to two sources: (1) biosynthetic variability in the assembly of the Lipid-A moiety and (2) loss of fatty acids from Lipid-A backbone during processing and purification. Consequently, it is difficult to control the manufacturing process in terms of reproducibility of composition of the mixture, which has significant bearing in biological activity and toxicity. For example, a reduction in adjuvant activity leads to reduction in immune response to an antigen that is formulated with Lipid-A as an adjuvant. The loss of a significant number of lipid chains during the processing of natural Lipid-A could result in the loss of adjuvant and other biological activities. Thus, it appears that lipid chains of Lipid-A molecules play a significant role in adjuvanticity such as internalization of antigens into macrophages and other antigen presenting cells (APC), leading to powerful immune responses.

While it is recognized that Lipid-A analogs are structurally complex, chemical synthesis is perhaps the best alternative to overcome the difficulties associated with accessing Lipid-A from

natural sources. Natural combination of lipids refers to the lipid diversity that exists in nature. There is no lipid diversity in the synthetic lipids of present invention as they carry a uniform of contingent of lipids, which are of similar carbon length. The present invention relates to the design and synthesis of some new mono-phosphorylated Lipid-A analogs, each carrying a combination of unnatural lipids, such as (I) and (II) (FIG. 3) or significantly, an unnatural combination of lipids (FIG. 4). The following features distinguish the synthetic Lipid-A structures disclosed in this invention from those obtained from natural sources and/or reported in the prior art in the field.

1) <u>Mono-phosphorylated</u>: A chemically unmodified Lipid-A structure from nature carries two phosphate groups at 1- and 4'-position, while the synthetic Lipid-A analogs in the present invention carry one phosphate group at 4'-position.
2) <u>A combination of unnatural lipids</u>: Molecules, such as compounds **33** and **102** (FIG. 3), contain at least one novel and unnatural lipid (I or II).
3) <u>An unnatural combination of lipids</u>: This refers to those Lipid-A analogs that carry lipids of uniform chain length, a combination that is not found in nature and their synthesis is not known in the prior art. Compounds **54** and **86** (FIG. 4) fall into this category. Compound **70** (FIG. 19) is similar, but it also contains an n-propyl group at 3-O-position and is an example of Lipid-A analog that incorporates a short unnatural alkyl group with an unnatural ether linkage.

All synthetic Lipid-A structures disclosed in this invention are expected to be mimics of naturally occurring *E. coli* derived and/or *Salmonella* derived Lipid-A structures (FIG. 1).

SUMMARY OF THE INVENTION

Though there are several publications detailing the minimal structure required for Lipid-A molecules for adjuvant activity with low toxicity, there has been no systematic study of structural features needed to maintain this activity.

A structurally defined molecule as an adjuvant is not commercially available for use with human therapeutic vaccines although some promising adjuvants are currently under clinical investigation. One example of such promising adjuvants is a natural Lipid-A product purified from bacterial cultures. The natural Lipid-A adjuvant product contains a mixture of several Lipid-A components with varying number of lipid chains. Lipid chain esters, which are attached to the carbohydrate core, can be cleaved during controlled hydrolysis of cell wall, leading to the formation of many components. One of the major problems associated with these preparations is the inconsistency in composition and performance as an adjuvant, the latter being highly critical to the effectiveness of vaccine based therapies. Other added factors such as high production costs and the difficulty in determining active ingredients in the final pharmaceutical composition render such adjuvants from natural sources commercially unattractive.

Synthetic Lipid-A analogs have several advantages over naturally derived adjuvant preparations. Synthetic compound is chemically defined with single structure and thus facilitates its tracking and control from manufacturing to final formulation. Synthetic product is cost effective and is easily adaptable for commercial scale-up while maintaining the consistency in both quality and performance.

Under the present invention novel Lipid-A analogs are designed, synthesized and finally incorporated into liposome vaccines containing the cancer associated mucin (MUC1) derived antigen, as a lipopeptide, to evaluate their adjuvanticity. Salient features of the present invention are described as follows.

New Lipid Structures

Though on any given natural Lipid-A structure the contingent of lipids are never of uniform length or structure, the most commonly found lipid in nature is (R)-3-hydroxy-tetradecanoic acid

(3-hydroxy myristic acid) and its 3-O-acylated derivatives. Lipid diversity contributes to by far the most significant variations among natural Lipid-A structures. While they are all linked through ester and amide bonds to the hydroxy and amino groups of the sugar respectively, variations include the number of lipids attached, the length of each lipid chain and the functional groups contained within the lipid chains. It is believed that these variations contribute to various biological functions of the entire Lipid-A molecule and more importantly to its adjuvant properties. Chemically speaking, ester linkages are labile as they are vulnerable to hydrolysis under physiological conditions. Gradual loss of lipid chains may slowly reduce the activity of the adjuvant under long storage of the vaccines thus diminishing their shelf life. Introduction of unnatural but stable ether linkages in place of esters, or combinations of both may enhance the stability of the adjuvant and may result in the longer shelf life for the vaccine formulations. A major advantage in the synthesis of a Lipid-A analog is that a molecule may be designed to achieve effectiveness as an adjuvant, safety and stability using the diversity in lipid chains and their linkages.

Incorporating these features, we have designed new synthetic lipid acids with general formulae (3) and (4) (FIG. 2) for building synthetic Lipid-A analogs. Compounds **5** and **23** were prepared in this invention as two specific examples of general structures (3) and (4). Compound **5** contains an aspartic acid moiety, which can be viewed as an β-amino acid. Its absolute configuration corresponds to (R)-3-hydroxy-tetradecanoic acid and thus compound **5** is considered to be a mimic of (R)-3-acyloxy-tetradecanoic acid. Compound **23** is a tri-lipid fatty acid containing an ether linkage, incorporated to enhance the stability of the whole molecule. Though ester based tri-lipid constructs have not so far been discovered among natural Lipid-A analogs, their presence may not be entirely excluded. The present invention also focuses on the synthesis of Lipid-A with a uniform lipid contingent in order to compare the adjuvant activities of those that exhibit lipid diversity.

Lipid-A Analogs with New Lipid Acid Attachments

Lipids of general formulae (3) and (4) (FIG. 2) are of new design and thus the corresponding Lipid-A structures that incorporate them are all distinctive. Two types of Lipid-A analogs,

monosaccharide (1) and disaccharide (2) (FIG. 3), have been designed and synthesized as part of the invention.

The monosaccharide derivative of structure (1) where at least one of R_1 and R_2 is independently chosen from structures (I) and (II) (FIG. 3) features the non-reducing end sugar of the natural Lipid-A structure. Compound **33** (FIG. 9) is an example of such structures.

And the disaccharide derivative of structure (2) where at least one of R_1, R_2, R_3, R_4 and R_5 is independently chosen from structures (I) and (II) (FIG. 3) is a monophosphorylated analog of natural Lipid-A structure. Compounds **58** (FIG. 16), **77** (FIG. 21), **102** (FIG. 29) and **104** (FIG. 30) are some examples with such structural features.

Lipid-A Analogs with Uniform Lipid Contingent

The lipid diversity of Lipid-A renders the molecule too complex and impractical for large-scale preparation through chemical synthesis. One of the main features of compounds designed as part of this invention is that the lipid substituents on 2-amino, 2'-amino, and 3'-O positions of the disaccharide backbone are identical and are composed of either ether or ester-linked di-lipid structure fragments. Such features are summarized in FIG. 4. The compound has the general formula (2) where R_1, R_4 and R_5 are identically having the di-lipid structure (III). Specific compounds are prepared in this invention as representative examples, such as structure **54** (FIG. 15), **70** (FIG. 19), **86** (FIG. 24) and **94** (FIG. 26).

A New Process for the Preparation of Lipid-A Analogs

The invention includes new processes for the synthesis of Lipid-A analogs (1) and (2). Disclosed herein are general synthetic routes to prepare variously substituted Lipid-A analogs of the invention. Different analogs can be easily obtained by using alternative starting materials. Details are illustrated in drawing figures and examples.

The process for the synthesis of monosaccharide derivative **33** is illustrated in FIG. 9 and the disaccharide derivative **48** in FIG. 10 – FIG. 13. An important feature of this new process is the general strategy of using combinations of different carbohydrate building blocks, protecting

group strategies and reagents to accomplish specific structures. For example, the 4,6-benzylidene protection on glucosamine derivative offers the freedom of selective ring opening to free the 4-OH on which the phosphate group may be introduced. Benzyl ester protected phosphate group, which is introduced through a two-step procedure, also offers the advantage of being easily deprotected, together with other benzyl groups on the molecule, at the final stage of the synthesis through catalytic hydrogenation. More examples are described in FIG. 14 – FIG. 21 for the synthesis of compounds **54**, **58**, **70** and **77**.

This process has been further modified to provide a more efficient procedure, which is particularly useful for the preparation of compounds with identical substituents on both amino groups of the carbohydrate backbone. Figures 22–24 illustrate the synthesis of compound **86** using this modified process.

In this modified process the phosphate group is introduced on the monosaccharide derivative before the glycosidic linkage is formed, and the glycosylation acceptor, which has both 4-OH and 6-OH groups unprotected (e.g. compound **79** in FIG. 22), is prepared through a simplified pathway. The steps involved in the whole process are reduced, especially at the disaccharide stage at which the material becomes more expensive and practically more difficult to handle. The process is designed for large-scale production and has been proven to be very efficient at gram-scale synthesis of a Lipid-A analog. Additional examples of compounds **94**, **102** and **104**, prepared using this modified process, are described in Figures 25–30.

The strategic intermediates disclosed in this invention are used in the synthesis of Lipid-A analogs and are not known in the literature.

Liposome Formulations

Liposomes are globular particles formed by the physical self-assembly of polar lipids, which define the membrane organization in liposomes. Liposomes may be formed as uni-lamellar or multi-lamellar vesicles of various sizes. Such liposomes, though constituted of small molecules having no immunogenic properties of their own, behave like macromolecular particles and display strong immunogenic characteristics.

Taking advantage of the self-assembling properties of lipids, one or more immunogens may be attached to the polar lipids that in turn become part of the liposome particle. Each immunogen comprises one or more antigenic determinants (epitopes). These epitopes may be B-cell epitopes (recognized by antibodies) or T-cell epitopes (recognized by T-cells). The liposome can act to adjuvant the immune response elicited by the associated immunogens. It is likely to be more effective than an adjuvant that is simply mixed with an immunogen, as it will have a higher local effective concentration.

Moreover, a hapten may be attached in place of the aforementioned immunogen. Like an immunogen, a hapten comprises an antigenic determinant, but by definition is too small to elicit an immune response on its own (typically, haptens are smaller than 5,000 daltons). In this case, the lipid moiety may act, not only as an adjuvant, but also as an immunogenic carrier, the conjugate of the hapten and the lipid acting as a synthetic immunogen (that is, a substance against which humoral and/or cellular immune responses may be elicited).

Even if the lipid does not act as an immunogenic carrier, the liposome borne hapten may still act as a synthetic antigen (that is, a substance which is recognized by a component of the humoral or cellular immune system, such as an antibody or T-cell). The term "antigen" includes both haptens and immunogens.

Thus, the invention contemplates a liposome whose membrane comprises a Lipid A analogue as disclosed herein, and at least one B-cell or T-cell epitope.

We have designed several synthetic antigens in the form of lipo-peptides, glyco-lipids and glyco-lipo-peptides that form the liposome membrane. Similarly, synthetic Lipid-A molecules of well-defined structural characteristics can be anchored into the liposome membrane.

Unlike the bacterial adjuvant preparations, a synthetic Lipid-A analog contributes a structurally well-defined lipids to the liposome membrane. Such defined structures not only reduce the

burden of re-affirming the 'active' membrane components after formulation, but also contribute to the definition of liposome membrane. Such liposomes may be designated as 'totally synthetic vaccine formulations' containing synthetic Lipid-A analog as an adjuvant and a synthetic lipopeptide as an antigen.

Epitope

The epitopes of the present invention may be B-cell or T-cell epitopes, and they may be of any chemical nature, including without limitation peptides, carbohydrates, lipids, glycopeptides and glycolipids. The epitope may be identical to a naturally occurring epitope, or a modified form of a naturally occurring epitope.

B-cell peptide epitopes are typically at least five amino acids, more often at least six amino acids, still more often at least seven or eight amino acids in length, and may be continuous ("linear") or discontinuous ("conformational") (the latter being formed by the folding of a protein to bring noncontiguous parts of the primary amino acid sequence into physical proximity). T-cell peptide epitopes are linear and usually 8 to 15, more often 9-11 amino acids in length.

Epitopes of interest include those specific to or otherwise associated with a pathogen, or a tumor. An epitope may be said to be associated with a particular infectious disease if it is presented by an intracellular, surface, or secreted antigen of the organism which causes the disease, or in the case of a virus, if it is associated with viral particles or is specific to a cell infected by the virus. It may be said to be associated with a particular tumor if it is presented by an intracellular, surface or secreted antigen of said tumor. It need not be presented by all cell lines of the tumor type in question, or by all cells of a particular tumor, or throughout the entire life of the tumor. It

need not be specific to the tumor in question. An epitope may be said to be "tumor associated" in general if it is so associated with any tumor (cancer, neoplasm).

The term "disease associated epitope" also includes any non-naturally occurring epitope which is sufficiently similar to an epitope naturally associated with the disease in question so that cytotoxic lymphocytes which recognize the natural disease epitope also recognize the similar non-natural epitope. Similar comments apply to "tumor associated epitope".

An epitope may be said to be specific to a particular source (such as a disease-causing organism or a tumor), if it is associated more frequently with that source than with other sources. Absolute specificity is not required, provided that a useful prophylactic, therapeutic or diagnostic effect is still obtained.

In the case of a "tumor-specific" epitope, it is more frequently associated with that tumor that with other tumors, or with normal cells. Preferably, there should be a statistically significant ($p=0.05$) difference between its frequency of occurrence in association with the tumor in question, and its frequency of occurrence in association with (a) normal cells of the type from which the tumor is derived, and (b) at least one other type of tumor. An epitope may be said to be "tumor-specific" in general is it is associated more frequently with tumors (of any or all types) than with normal cells. It need not be associated with all tumors.

The term "tumor specific epitope" also includes any non-naturally occurring epitope which is sufficiently similar to a naturally occurring epitope specific to the tumor in question (or as appropriate, specific to tumors in general) so that cytotoxic lymphocytes stimulated by the similar epitope will be essentially as specific as CTLs stimulated by the natural epitope.

In general, tumor-versus-normal specificity is more important than tumor-versus-tumor specificity as (depending on the route of administration and the particular normal tissue affected), higher specificity generally leads to fewer adverse effects. Tumor-versus-tumor specificity is more important in diagnostic as opposed to therapeutic uses.

The reference to a CTL epitope as being "restricted" by a particular allele of MHC, such as HLA-A1, indicates that such epitope is bound and presented by the allelic form in question. It does not mean that said epitope might not also be bound and presented by a different allelic form of MHC, such as HLA-A2, HLA-A3, HLA-B7, or HLA-B44.

The term "specific" is not intended to connote absolute specificity, merely a clinically useful difference in probability of occurrence in association with a pathogen or tumor rather than in a matched normal subject.

Pathogens may be submicrobial (e.g., viruses), microbial (e.g., fungi, protozoa), or multicellular (e.g, worms, arthropods, etc.). Tumors may be of mesenchymal or epithelial origin. Cancers include cancers of the colon, rectum, cervix, breast, lung, stomach, uterus, skin, mouth, tung, lips, larynx, kidney, bladder, prostate, brain, and blood cells.

Naturally occurring epitopes may be identified by a divide-and-test process. One starts with a protein known to be antigenic or immunogenic. One next tests fragments of the protein for immunological activity. These fragments may be obtained by treatment of the protein with a proteolytic agent, or, if the peptide sequence is known, one may synthetically prepare smaller peptides corresponding to subsequences of the protein. The tested fragments may span the entire protein sequence, or just a portion thereof, and they may be abutting, overlapping, or separated. If any of the fragments are immunologically active, the active fragments may themselves be subjected to a divide-and-test analysis, and the process may be continued until the minimal length immunologically active sequences are identified. This approach may be used to identify either B-cell or T-cell epitopes, although the assays will of course be different. Geysen teaches systematically screening all possible oligopeptide (pref. 6-10 a.a.) abutting or overlapping fragments of a particular protein for immunological activity in order to identify linear epitopes. See WO 84/03564.

It is also possible to predict the location of B-cell or T-cell peptide epitopes if an amino acid sequence is available. B-cell epitopes tend to be in regions of high local average hydrophilicity. See Hopp and Wood, Proc. Nat. Acad. Sci. (USA) 78: 3824 (1981); Jameson and Wolf, CABIOS, 4: 181 (1988). T-cell epitopes can be predicted on the basis of known consensus sequences for the peptides bound to MHC class I molecules of cells of a particular haplotype. See e.g., Slingluff, WO98/33810, especially pp. 15-16; Parker, et al., "Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side chains", J. Immunol. 152: 163 (1994).

Naturally occurring T-cell epitopes may be recovered by dissociating them from their complexes with MHC class I molecules and then sequencing them, e.g., by mass spectroscopic techniques.

Generally speaking, in addition to epitopes which are identical to the naturally occurring

disease- or tumor-specific epitopes, the present invention embraces epitopes which are different from but substantially identical with such epitopes, and therefore disease- or tumor-specific in their own right. It also includes epitopes which are not substantial identical to a naturally occurring epitope, but which are nonetheless cross-reactive with the latter as a result of a similarity in 3D conformation.

An epitope is considered substantially identical to a reference epitope (e.g., a naturally occurring epitope) if it has at least 10% of an immunological activity of the reference epitope and differs from the reference epitope by no more than one non-conservative substitution.

If it is a CTL epitope, it may incorporate further nonconservative substitutions which are suggested by a known binding motif of the pertinent MHC molecule. Kast, et al., J. Immunol, 152:3904-12 (1994) sets forth HLA-A specific peptide binding motifs for the HLA molecules A1, A2.1, A3, A11 and A24. Engelhard, et al., in Sette, ed., Naturally Processed Peptides, 57:39-62 (1993) explored the features that determined binding to HLA-A2.1 and HLA-B7. See also Hobohim et al; Eur. J. Immunol., 23:1271-6 (1993); Kawakami, et al., J. Immunol., 154:3961-8 (1995). Based on these and other sources, the preferred and tolerated AAs for various HLA molecules include (but are not limited to) the following:

Table 10

Molecule	Position	Preferred AA	tolerated AA
A1	2	T, S, M	
	3	D, E	A, S
	9	Y	
A2.1	2	L, M	I, V, A, T
	9	L, V, I	A, M, T
A3	2	L, M, I, V, S A, T, F	C, G, D
	9	K, R, Y, H, F	A
A11	2	M, L, I, V, S A, T, G, N	C, D, F
	9	K	R, H, Y
A24	2	Y, F, W	M
	9	F, L, I, W	
B7	1	A	M, S, R, L
	2	P	V
	3	R	A, K, S, M
	9	L	I, A, V
B8	3	K	not known

	5	K	not known
	9	L	not known
B27	2	R	not known
	9	R, K, H	not known
B35	2	P	not known
	9	Y	not known
B53	2	P	not known

If a position is not listed, studies revealed a greater variability of AAs than for the listed positions. For listed positions, AAs not listed may be tolerated, especially if they are conservative or semi-conservative substitutions for "preferred" or "tolerated" AAs.

Conservative substitutions are herein defined as exchanges within one of the following five groups:

I. Small aliphatic, nonpolar or slightly polar residues:
Ala, Ser, Thr, Pro, Gly

II. Polar, negatively charged residues: and their amides
Asp, Asn, Glu, Gln

III. Polar, positively charged residues:
His, Arg, Lys

IV. Large, aliphatic, nonpolar residues:
Met, Leu, Ile, Val, Cys

V. Large, aromatic residues:
Phe, Tyr, Trp

Within the foregoing groups, the following substitutions are considered "highly conservative":

Asp/Glu
His/Arg/Lys
Phe/Tyr/Trp
Met/Leu/Ile/Val

Semi-conservative substitutions are defined to be exchanges between two of groups (I)-(V) above which are limited to supergroup (A), comprising (I), (II) and (III) above, or to supergroup (B), comprising (IV) and (V) above. Also, Ala is considered a semi-conservative substitution for all non group I amino acids.

It will be appreciated that highly conservative substitutions are less likely to affect activity than other conservative substitutions, conservative substitutions are less likely to affect activity than merely semi-conservative substitutions, and semi-conservative substitutions less so

than other non-conservative substitutions. In addition, single substitutions are less likely to affect activity than are multiple mutations.

Although a substitution mutant, either single or multiple, of the peptides of interest may not have quite the potency of the original peptide, such a mutant may well be useful.

Substitutions are not limited to the genetically encoded, or even the naturally occurring amino acids. When the epitope is prepared by peptide synthesis, the desired amino acid may be used directly. Alternatively, a genetically encoded amino acid may be modified by reacting it with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues.

A non-genetically encoded amino acid is considered a conservative substitution for a genetically encoded amino acid if it is more similar in size (volume) and hydrophilicity to the original amino acid, and to other amino acids in the same exchange group, than it is to genetically encoded amino acids belonging to other exchange groups.

Substantially identical peptide epitopes may be identified by a variety of techniques, some of which do not depend on preexisting knowledge of the binding motif. Thus, it is known in the art that one may synthesize all possible single substitution mutants of a known peptide epitope. For a nonpeptide, there are (20x9-1=179) such mutants. Geysen, et al., Proc Nat. Acad. Sci. (USA), 81:3998-4002 (1984). While the effects of different substitutions are not always additive, it is reasonable to expect that two favorable or neutral single substitutions at different residue positions in the epitope can safely be combined in most cases.

Both naturally occurring and non-naturally occurring peptide epitopes may be identified, if a suitable antibody or other receptor is available, by screening a peptide combinatorial library for peptides bound by the target. Humoral peptide epitopes may be identified by screening a combinatorial peptide phage library for specific binding to a target monoclonal antibody known to recognize the antigen of interest. Preferably, the library is prescreened to eliminate peptides which bind the antibody other than at the epitope binding site of the antibody; this can be done by eliminating phage which bind to a second, control antibody of the same isotype.

Similarly, to identify CTL peptide epitopes, one may synthesize a family of related single or multiple substitution mutants, present the mixture to the HLA-A2.1 positive lymphoblastoid cell line T2 (or other cell line capable of presenting specific CTL epitopes), and expose the T2 cells to CTLs of the desired specificity. If the T2 cells are lysed, the effective epitopes may be identified either by direct recovery from the T2 cells or by a progressive process of testing subsets of the effective peptide mixtures. Methods for the preparation of degenerate peptides are described in Rutter, USP 5,010,175, Haughten, et al., Proc. Nat. Acad. Sci. (USA), 82:5131-35 (1985), Geysen, et al., Proc. Nat. Acad. Sci. (USA), 81:3998-4002 (1984); W086/06487; W086/00991.

Multiple mutagenesis may be used to screen a few residue positions intensely or a larger number of positions more diffusely. One approach is to explore at least a representative member of each a.a. type at each position, e.g., one representative of each of exchange groups I-V as hereafter defined. Preferably, Gly and Pro are screened in addition to one other group I residue. Preferably, at least one screened residue is an H-bonding residue. If a positive mutant features a particular representative, like amino acids can be explored in a subsequent library. If, for example, a Phe substitution improves binding, Tyr and Trp can be examined in the next round.

The person of ordinary skill in the art, in determining which residues to vary, may also make comparisons of the sequences of the naturally processed MHC associated peptides, and may obtain 3D structures of the MHC: peptide: TCR complexes, in order to identify residues involved in MHC or TCR binding. Such residues may either be left alone, or judiciously mutated in an attempt to enhance MHC or TCR binding.

An extensive discussion of carbohydrate haptens appears in Wong, USP 6,013,779.

Adjuvanticity of Lipid-A Analogs

It is generally understood that a synthetic antigen of low molecular weight is weakly immunogenic, which is the biggest obstacle to the success of a fully synthetic vaccine. One way to improve the imunogenicity of such a synthetic antigen is to deliver it in the environment of an adjuvant. The primary target of those new synthetic Lipid-A analogs in the present invention is their adjuvant properties. An ideal adjuvant is believed to non-specifically stimulate the immune system of the host, which upon the subsequent encounter of any foreign antigen can produce strong and specific immune response to that foreign antigen. Such strong and specific immune response, which is also characterized by its memory, can be produced only when T-lymphocytes (T-cells) of the host immune system are activated. Here we choose T-cell blastogenesis and IFN-γ production as two important parameters for measuring the immune response.

Experimentally T-cell blastogenesis measures DNA synthesis that directly relates to T-cell proliferation, which in turn is the direct result of the T-cell activation. On the other hand, IFN-γ is a major cytokine secreted by T-cells when they are activated. Therefore, both T-cell blastogenesis and IFN-γ production indicate T-cell activation, which suggests the ability of an adjuvant in helping the host immune system to induce a strong and specific immune response to any protein-based antigen. By using a synthetic lipopeptide antigen, H_2N-STAPPAHGVTSAPDTRPAPGSTAPPK(Pal)G-OH (FIG. 34, single letter amino acid codes are defined in Table 8,), a modified 25-amino-acid sequence that is derived from tumor-associated MUC1 mucin, we were able to evaluate the adjuvant properties of the synthetic Lipid-A analogs disclosed in this invention. Based on the data of T-cell blastogenesis and IFN-γ level (Figures 31–33) obtained through preliminary *in vivo / in vitro* studies, it is amply demonstrated that

synthetic Lipid-A structures **48, 54, 70, 86, 102** and **104** are as effective, as adjuvants, as the Lipid-A preparations of bacterial origin.

The compound is considered an adjuvant if it significantly (p=0.05) increases the level of either T-cell blastogenesis or of interferon gamma production in response to at least one liposome/immunogen combination relative to the level elicited by the immunogen alone. Preferably, it does both. Preferably, the increase is at least 10% , more preferably at least 50%, still more preferably, at least 100%.

Preliminary *in vivo* toxicity evaluation of synthetic Lipid-A analog **86** has shown that its toxicity is much lower than that of natural Lipid-A product obtained from bacteria *Salmonella* (Table 4, **Example 99**). Thus, there are many advantages associated with the totally synthetic and novel Lipid-A structures disclosed in this invention, in terms of efficacy, safety, stability and compliance of such vaccine formulations with regulatory guidelines.

Preferably, the toxicity of the lipid compounds of the present invention is not more than 50% that of said natural Lipid-A product; more preferably it is less than 10% that of the latter.
The *in vivo* studies of the synthetic compounds disclosed in this invention have been limited to the assessment of their effectiveness as adjuvant. But they may have broader applications in other areas such as anti-tumor agents, LPS / Lipid-A antagonists, inhibitors for Lipid-A biosynthesis and thus useful as novel antibiotics. Results of various biological activities will be disclosed in due course.

Pharmaceutical Methods and Preparations

Applicants hereby incorporate by reference the discussion at pp. 32-46 of WO98/33810.

The preferred animal subject of the present invention is a primate mammal. By the term "mammal" is meant an individual belonging to the class Mammalia, which, of course, includes humans. The invention is particularly useful in the treatment of human subjects, although it is intended for veterinary uses as well. By the term "non-human primate" is intended any member of the suborder Anthropoidea except for the family Hominidae. Such non-human primates include the superfamily Ceboidea, family Cebidae (the New World monkeys including the

capuchins, howlers, spider monkeys and squirrel monkeys) and family Callithricidae (including the marmosets); the superfamily Cercopithecoidea, family Cercopithecidae (including the macaques, mandrills, baboons, proboscis monkeys, mona monkeys, and the sacred hunaman monkeys of India); and superfamily Hominoidae, family Pongidae (including gibbons, orangutans, gorillas, and chimpanzees). The rhesus monkey is one member of the macaques.

The term "protection", as used herein, is intended to include "prevention," "suppression" and "treatment." "Prevention" involves administration of the protein prior to the induction of the disease. "Suppression" involves administration of the composition prior to the clinical appearance of the disease. "Treatment" involves administration of the protective composition after the appearance of the disease.

It will be understood that in human and veterinary medicine, it is not always possible to distinguish between "preventing" and "suppressing" since the ultimate inductive event or events may be unknown, latent, or the patient is not ascertained until well after the occurrence of the event or events. Therefore, it is common to use the term "prophylaxis" as distinct from "treatment" to encompass both "preventing" and "suppressing" as defined herein. The term "protection," as used herein, is meant to include "prophylaxis." It should also be understood that to be useful, the protection provided need not be absolute, provided that it is sufficient to carry clinical value. An agent which provides protection to a lesser degree than do competitive agents may still be of value if the other agents are ineffective for a particular individual, if it can be used in combination with other agents to enhance the level of protection, or if it is safer than competitive agents.

The composition may be administered parentally or orally, and, if parentally, either systemically or topically. Parenteral routes include subcutaneous, intravenous intradermal, intramuscular, intraperitoneal, intranasal, transdermal, or buccal routes. One or more such routes may be employed. Parenteral administration can be, e.g., by bolus injection or by gradual perfusion over time. Alternatively, or concurrently, administration may be by the oral route. The immunization is preferably accomplished initially by intramuscular injection followed by intradermal injection, although any combination of intradermal and intramuscular injections may be used.

It is understood that the suitable dosage of a immunogen of the present invention will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. However, the most preferred dosage can be tailored to the individual subject, as is understood and determinable by one of skill in the art, without undue experimentation. This will typically involve adjustment of a standard dose, e.g., reduction of the dose if the patient has a low body weight.

Prior to use in humans, a drug will first be evaluated for safety and efficacy in laboratory

animals. In human clinical studies, one would begin with a dose expected to be safe in humans, based on the preclinical data for the drug in question, and on customary doses for analogous drugs (if any). If this dose is effective, the dosage may be decreased, to determine the minimum effective dose, if desired. If this dose is ineffective, it will be cautiously increased, with the patients monitored for signs of side effects. See, e.g., Berkow, et al., eds., The Merck Manual, 15th edition, Merck and Co., Rahway, N.J., 1987; Goodman, et al., eds., Goodman and Gilman's The Pharmacological Basis of Therapeutics, 8th edition, Pergamon Press, Inc., Elmsford, N.Y., (1990); Avery's Drug Treatment: Principles and Practice of Clinical Pharmacology and Therapeutics, 3rd edition, ADIS Press, LTD., Williams and Wilkins, Baltimore, MD. (1987), Ebadi, Pharmacology, Little, Brown and Co., Boston, (1985), which references and references cited therein, are entirely incorporated herein by reference.

The total dose required for each treatment may be administered in multiple doses (which may be the same or different) or in a single dose, according to an immunization schedule, which may be predetermined or ad hoc. The schedule is selected so as to be immunologically effective, i.e., so as to be sufficient to elicit an effective immune response to the antigen and thereby, possibly in conjunction with other agents, to provide protection. The doses adequate to accomplish this are defined as "therapeutically effective doses." (Note that a schedule may be immunologically effective even though an individual dose, if administered by itself, would not be effective, and the meaning of "therapeutically effective dose" is best interpreted in the context of the immunization schedule.) Amounts effective for this use will depend on, e.g., the peptide composition, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician.

Typically, the daily dose of an active ingredient of a pharmaceutical, for a 70 kg adult human, is in the range of 10 nanograms to 10 grams. For immunogens, a more typical daily dose for such a patient is in the range of 10 nanograms to 10 milligrams, more likely 1 microgram to 10 milligrams. However, the invention is not limited to these dosage ranges.

It must be kept in mind that the compositions of the present invention may generally be employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, in view of the minimization of extraneous substances and the relative nontoxic nature of the peptides, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these peptide compositions.

The doses may be given at any intervals which are effective. If the interval is too short,

immunoparalysis or other adverse effects can occur. If the interval is too long, immunity may suffer. The optimum interval may be longer if the individual doses are larger. Typical intervals are 1 week, 2 weeks, 4 weeks (or one month), 6 weeks, 8 weeks (or two months) and one year. The appropriateness of administering additional doses, and of increasing or decreasing the interval, may be reevaluated on a continuing basis, in view of the patient's immunocompetence (e.g., the level of antibodies to relevant antigens).

A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al., Ann. Rev. Biophys. Bioeng. 9:467 (1980), U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019369, incorporated herein by reference.

The appropriate dosage form will depend on the disease, the immunogen, and the mode of administration; possibilities include tablets, capsules, lozenges, dental pastes, suppositories, inhalants, solutions, ointments and parenteral depots. See, e.g., Berker, supra, Goodman, supra, Avery, supra and Ebadi, supra, which are entirely incorporated herein by reference, including all references cited therein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1	Examples of natural Lipid-A structures, **A** from *E. coli* and **B** from *Salmonella* strains
FIG. 2	New lipid acids of general formulae (3) and (4) and two examples thereof
FIG. 3	Monosaccharide and disaccharide Lipid-A analogs containing new lipid structures and two examples thereof
FIG. 4	Disaccharide Lipid-A analogs containing uniform di-lipid chains and two examples thereof
FIG. 5	Synthesis of lipid **5**
FIG. 6	Synthesis of lipids
FIG. 7	Synthesis of tri-lipid **23**
FIG. 8	Synthesis of glucosamine derivatives
FIG. 9	Synthesis of compound **33**
FIG. 10	Synthesis of glycosylation acceptors
FIG. 11	Synthesis of glycosylation donor **43**
FIG. 12	Synthesis of disaccharide **45**
FIG. 13	Synthesis of compound **48**
FIG. 14	Synthesis of disaccharide **51**
FIG. 15	Synthesis of compound **54**
FIG. 16	Synthesis of compound **58**
FIG. 17	Synthesis of glycosylation acceptor **64**
FIG. 18	Synthesis of disaccharide **67**
FIG. 19	Synthesis of compound **70**
FIG. 20	Synthesis of amine **73**
FIG. 21	Synthesis of compound **77**
FIG. 22	Synthesis of glycosylation **79**
FIG. 23	Synthesis of glycosylation donor **83**
FIG. 24	Synthesis of compound **86**
FIG. 25	Synthesis of glycosylation donor **91**
FIG. 26	Synthesis of compound **94**

FIG. 27 Synthesis of glycosylation acceptor **98**

FIG. 28 Synthesis of amine **100**

FIG. 29 Synthesis of compound **102**

FIG. 30 Synthesis of compound **104**

FIG. 31 T-cell proliferation (CPM, counts per minute) and Interferon-gamma (IFN-γ, pg/ml) production in mice immunized with MUC1-based liposomal vaccine adjuvanted with Lipid-A analogs **33, 48, 58** or **Natural Lipid-A**

FIG. 32 T-cell proliferation (CPM, counts per minute) and Interferon-gamma (IFN-γ, pg/ml) production in mice immunized with MUC1-based liposomal vaccine adjuvanted with Lipid-A analogs **48, 54, 70, 77, 86** or **Natural Lipid-A**

FIG. 33 T-cell proliferation (CPM, counts per minute) and Interferon-gamma (IFN-γ, pg/ml) production in mice immunized with MUC1-based liposomal vaccine adjuvanted with Lipid-A analogs **86, 94, 102, 104** or **Natural Lipid-A**

FIG. 34 Structure of lipopeptide BP1-148, a modified 25-amino-acid sequence derived from tumor-associated MUC1 mucin (single letter codes of amino acids)

FIG. 35 Examples of peptides and glycopeptides derived from viral and tumor antigens that can be incorporated into liposome formulations.

DETAILED DESCRIPTIONS OF THE PREFERRED EMBODIMENTS OF THE INVENTION

DETAILED DESCRIPTION OF THE FIGURES

Chemical Synthesis of Lipid-A Analogs (FIG. 5 – FIG. 30)

An invariant structural feature of Lipid-A molecule is its β-(1,6)-linked D-glucosamine disaccharide backbone. While this structure is bacterial in origin, this disaccharide in its pure form probably has no relevance to the range of biological activities displayed by the lipid structure. The lipid chains perhaps provide the basic mechanism through which the disaccharide exhibits a wide range of properties. Thus, only the lipid chains play a quantitative role through the promotion of the Lipid-A properties. A wide range of lipids may fill this need, while only in their numbers the lipids seem to quantitatively influence the properties of the molecule. We have synthesized and examined the influence of a range of lipids both natural (compound **13–15** and **17** in FIG. 6) and unnatural (**5** in FIG. 5, **20** and **23** in FIG. 7) and a range of linkages through which the lipids are linked together and to the carbohydrate core.

The most commonly found lipid in Lipid-A structures from nature is (R)-3-hydroxy-myristic acid. The 3-hydroxy group is further acylated with lipids of different lengths. Compound **5** (FIG. 5) is designed employing the natural stereochemistry of aspartic acid. Since the three dimensional orientation of the amino group in **5** is identical to that of the hydroxyl group in (R)-3-hydroxy myristic acid, **5** is expected to be a stereostructural mimic of (R)-3-acyloxy myristic acid.

The preparation of **5** is described in FIG. 5. Commercially available aspartic acid derivative **1** was coupled with nonylamine by using isobutyl chloroformate as an activating reagent to obtain **2** in 79% yield. The removal of Fmoc group from **2** followed by coupling with tetradecanoic acid provided **4**. The *tert*-butyl protecting group was removed by treatment with TFA to give **5** in 97% yield.

FIG. 6 describes the synthesis of (R)-3-hydroxy-myristic acid derivative. Reformatsky reaction[20] of dodecanal with ethyl bromoacetate gave **6**, which was hydrolyzed to racemic 3-hydroxymyristic acid **7**. Optical resolution of **7** was achieved according to the known procedure[21a, b]. The diastereomers of its dehydroabietylamine salt were separated through fractional crystallization and the diastereomeric purity of the salt was monitored by melting point and/or NMR spectroscopy. Base and acid treatment of the salt gave R-isomer **8** in >95% enantiomeric excess (e.e.), which was converted to phenacyl ester **9** in 92% yield. Acylation of the 3-hydroxy group with various acyl chlorides in pyridine in the presence of DMAP gave **10**–**12** which upon treatment with zinc powder gave free lipid acids **13** – **15**[22, 23]. On the other hand **9** was treated with benzyl trichloroacetimidate in the presence of trifluoromethyl sulphonic acid to give the 3-benzyloxy derivative **16** which was subsequently converted to its free acid **17**[24].

FIG. 7 illustrates the synthesis of novel di-lipid **20** and tri-lipid **23** containing unnatural ether linkage. The reaction of **9** with triflate **18** in the presence of potassium carbonate gave **19** which was de-protected to give the di-lipid acid **20**. Asymmetric synthesis of **21**[25a, b, c] from dodecanal and allyl bromide in the presence of chiral reagent (-)-DIPCl at –78°C afforded the product with ~85% e.e. in about 70% yield. The enantiomeric excess was determined from its optical rotation data and the NMR data of its methyl trifluoromethyl phenyl acetic acid [(R)-(+)-MTPA] ester[26, 27]. **21** and **20** were coupled in the presence of DCC and DMAP in dichloromethane to form **22** in 81% yield. Oxidative cleavage of the double bond in **22** gave the tri-lipid free acid **23** in 61% yield. Among the reported Lipid-A structures from nature, the presence of a tri-lipid moiety is not yet known.

FIG. 8 shows the general procedure used to prepare the glucosamine derivatives. These derivatives are well known and they are prepared essentially according to the procedures described in literatures[28, 29].

In FIG. 9 the synthesis of a monosaccharide analog **33** of the non-reducing end of Lipid-A structure is described. Compound **26** was coupled with tetradecanoic acid in the presence DCC and DMAP to afford **29** in 91% yield. The reductive removal of Troc-group in **29** with zinc powder in acetic acid, followed by coupling with lipid acid **5**, furnished compound **30** in 60% yield. Regioselective benzylidene-ring opening with sodium cyanoborohydride and etherial HCl

solution afforded the 4-OH compound **31** in 86%. A two step phosphate group introduction[30] was effected by reacting **31** with dibenzyl diisopropylphsophoramidite to form phosphite, followed by oxidation with m-chloroperbenzoic acid (m-CPBA) to give **32** in 66% yield. Catalytic hydrogenation to remove benzyl protecting groups of **32** gave the monosaccharide Lipid-A analog **33** in 79% yield.

FIG. 10 describes the preparation of two glycosylation acceptors to be used in the synthesis of monophosphorylated disaccharide Lipid-A analogs. The glucosamine derivative **34**[31] was readily available from glucosamine hydrogen chloride. Regioselective introduction of trityl group in 6-O-position of **34** in the presence to DMAP and pyridine at 40°C gave **35** in 90% yield. Benzylation with benzyl bromide and sodium hydride, followed by treatment with 80% acetic acid at 110°C gave **37**. The phthalimido group was removed by treatment with hydrazine in ethanol at reflux to give free amine **38**, which was coupled in the presence of DCC with lipid acids **15** and **14** to provide **39** and **40**, respectively.

The preparation of glycosylation donor **43** is shown in FIG. 11. Compound **28** was coupled with **14** in the presence of DCC and DMAP to obtain **41** in very good yield. Allyl group was then removed in two steps, first the isomerization of allyl double bond by treating with Ir(I) complex and then hydrolysis in the presence of NBS. Treatment of **42** with trichloroacetonitrile and DBU afforded **43** in 81% yield.

The complete synthesis for disaccharide Lipid-A analog **48** is illustrated in FIG. 12 and FIG. 13. The glycosylation reaction between **39** and **43** with trifluoroboron diethyl etherate as the catalyst furnished the desired disaccharide **44** in 65% yield (FIG. 12). The Troc-protection group in **43**, which served as a neighbour participating group, controlled the outcome of the stereochemistry of this glycosylation reaction to provide β-glycoside, exclusively. The removal of Troc-group in **44**, followed by coupling with lipid acid **13** in the presence of DCC, gave compound **45**. The selective benzylidene ring opening with sodium cyanoborohydride / HCl provides the 4'-OH compound **46** (FIG. 13). The phosphate group was introduced through phosphite formation and subsequent oxidation giving **47** in 85% yield. Final debenzylation with palladium on charcoal and hydrogen in THF-acetic acid afforded the monophosphorylated Lipid-A analog **48**.

The whole strategy described above is very efficient in constructing disaccharide Lipid-A analogs, and it is also very flexible to adapt variations in the lipid contingent by using a few common building blocks. The same strategy was used to prepare two other analogs **54** and **58**, which are described in FIG. 14 - FIG. 16.

Compared to the proposed structure of *Salmonella*-type Lipid-A (FIG. 1), compound **48** (FIG. 13) has no lipid attached at the 3-O-position while the rest of the lipid substitution pattern remains the same. Compound **54** (FIG. 15) has the same substitution pattern as **48** except that in **54** all the lipids are of identical length (C_{14}). The repetition of lipids can be advantageous for large-scale preparation of the compound. Compound **58** (FIG. 16) carries an unnatural aspartic acid based lipid, but it may be considered as a close mimic of **54**.

In order to introduce another lipid acid to 3-O-position of the disaccharide backbone, a different protecting strategy in the reducing end sugar has been employed. FIG. 17 shows the preparation of a new glycosylation acceptor **64**. From **59**, 3-O-allylation, removal of benzylidene group, and regioselective introduction of a trityl group gave **60**. Benzylation of 4-OH group in **60** with benzyl bromide and sodium hydride, followed by the removal of trityl group afforded **62**. The phthalimido group in **62** was cleaved by treatment with hydrazine in refluxing ethanol giving free amine compound **63** which was acylated with **14** in the presence of DCC to produce **64**. The allyl group at 3-O-position provides the freedom of introducing a lipid to 3-O-position at any stage during the synthesis and if not removed, the allyl group will undergo catalytic hydrogenation to n-propyl group and it remains as a permanent appendage to the molecule.

Using the same procedure as described for the synthesis of compound **48**, coupling of **43** and **64** gave the desired disaccharide **65** in 88% yield (FIG. 18). Through reductive cleavage of Troc-group, acylation of the free amine, **65** was converted to **67** which finally led to Lipid-A analog **70** after a series of transformations including reductive benzylidene ring opening, phosphate group introduction and hydrogenation (FIG. 19). Analog **70** has an n-propyl group on 3-O-position connected through an ether linkage. Compounds with such short simple alkyl chain

connected through an ether linkage have not been reported before for Lipid-A analogs either from natural sources or through chemical synthesis.

FIG. 20 illustrates the strategy of introducing a lipid acid at 3-O-position of the disaccharide backbone. The allyl group of **65** was removed by treatment with Ir(I) complex, followed by NBS to give **71**. The coupling of **71** with **17** in the presence of DCC and DMAP gave **72** in 71% yield, which following the treatment with zinc in acetic acid formed the free amine **73**. By carrying out the same series of reactions as described above, compound **73** was converted to the final structure **77** (FIG. 21)

In the process of preparing several other Lipid-A analogs a new simplified strategy has been adapted for the synthesis of **86** as shown in FIG. 22 - FIG. 24. In FIG. 22 the glycosyl acceptor **79** was prepared in two steps from **27** through acylation at 3-O-position and removal of 4, 6-isopropylidene group.

FIG. 23 describes the preparation of new glycosyl donor **83** which is complete with benzyl protected phosphate group at 4-O-position. Selective opening of benzylidene ring in **41** using sodium cyanoborohydride and dry HCl gave compound **80** in good yield. Benzyl protected phosphate group was then introduced into 4-O-position to form **81** in 85% yield. De-allylation followed by the reaction with trichloroacetonitrile and DBU formed the new glycosylation donor **83**.

The coupling reaction between **79** and **83** (FIG. 24) was carried out in the presence of trifluoroboron etherate as catalyst to form the desired disaccharide **84** in 66% yield. Reductive removal of Troc-groups, followed by the coupling with lipid acid **14**, afforded compound **85** which, following debenzylation, gave the Lipid-A analog **86**.

In the modified strategy, the phosphate group was introduced before the formation of glycosidic linkage. The glycosylation of acceptor with two free hydroxy groups proceeded with both regioselectivity and stereoselectivity forming only (1→6)-connected-β-glycoside. In doing so the synthetic steps were reduced at the disaccharide stage. This process becomes very efficient for

Lipid-A analogs with identical substituents on 2- and 2'-amines, which may be introduced simultaneously.

Using this same procedure another Lipid-A analog **94** was prepared (FIG. 25, FIG. 26). The difference between compounds **86** and **94** is that in the latter the di-lipid moieties contain ether linkages. Though the ether linkage is not known in naturally derived Lipid-A structures, it confers better stability to Lipid-A structures than the natural ester linkage. In addition, the ether linkage is not susceptible to hydrolytic conditions and is more hydrophobic. Consequently, the lipid moiety with ether linkage attains better stability and may display better adjuvant characteristics than its natural counterpart. In FIG. 25 the preparation of glycosylation donor **91** is described. Starting with the coupling of **28** with lipid acid **20** (→**87**), through benzylidene ring opening (→**88**), phosphate group introduction (→**89**), de-allylation (→**90**) and imidate formation, compound **91** was obtained. As described above the glycosylation reaction of **79** and **91** furnished the disaccharide **92** (FIG. 26), which through the Troc-group removal, acylation with lipid acid **20**, and final debenzylation, afforded Lipid-A analog **94**.

Using the modified strategy, two more synthetic examples of Lipid-A analogs are described in FIG. 27 - FIG. 30. In FIG. 27 the preparation of a new glycosylation acceptor **98** is described. A benzyl group was introduced at the 3-O-position of **27** using benzyl trichloroacetimidate and trifluoromethyl sulfonic acid to provide **95**. Both the isopropylidene and Troc-group were removed to form the free amine **97**, which upon coupling with the tri-lipid acid **23** afforded the glycosyl acceptor **98**.

The glycosylation reaction between **91** and **98** in the presence of trifluoroboron etherate gave the desired disaccharide **99** in 56% yield (FIG. 28), which upon treatment with activated zinc in acetic acid provided the amino compound **100**. Acylation of the amino-group with **20** (FIG. 29) and **17** (FIG. 30) gave **101** and **103**, respectively. Complete de-protection of **101** and **103** furnished two Lipid-A analogs **102** and **104**, respectively.

Both compound **102** and **104** carry tri-lipid moieties on the amino-group at the reducing end sugar. There have been no reports of naturally occurring Lipid-A structures with tri-lipid

components nor have any chemically synthesized structures been reported. It is believed that the number of lipid chains attached to the carbohydrate core quantitatively modulates the biological activities of Lipid-A analogs. Whether the presence of a tri-lipid moiety on Lipid-A structure has a net quantitative effect on biological activities still remains to be investigated. We believe that the tri-lipid structural fragment does not significantly alter biological properties of Lipid-A structures. Our preliminary *in vivo* test results show that both compounds **102** and **104** have exhibited adjuvant characteristics similar to those of Lipid-A preparation of bacterial origin as well as to those of synthetic analog **86**.

Biological Test Results (Figures 31–33)

Liposome formulations have been used to evaluate the adjuvant properties of various synthetic Lipid-A structures and the immune responses to a synthetic lipopeptide antigen BP-148, H_2N-STAPPAHGVTSAPDTRPAPGSTAPPK(Pal)G-OH (FIG. 34), a modified 25-amino-acid sequence derived from tumor-associated MUC1 mucin. The commercially available Lipid-A product, which is currently under clinical evaluation as an adjuvant for vaccine programs, was used for comparison. The natural Lipid-A product, purchased from AVANTI, contained a mixture of Lipid-A analogs extracted from *Salmonella* bacterial cell wall and is designated as **Natural Lipid-A** in this invention.

Thus, the liposomal formulations containing the synthetic lipopeptide antigen, BP1-148, and Lipid-A analogs **33, 48, 54, 58, 70, 77, 86, 94, 102, 104** or **Natural Lipid-A**, were used to immunize mice to measure their immune response in terms of T-cell blastogenesis and interferon-gamma (IFN-γ) production. The compounds were tested in three groups at different time and their preliminary results are shown separately in Figures 31–33. As the variations are attributed to different factors such as animal groups and experimental factors, the data from different groups will not be compared.

T-cell blastogenesis and high levels of IFN-γ production in mice after the introduction of such liposomal formulations amply demonstrated that synthetic Lipid-A structures **48, 54, 70, 86, 102** and **104** have similar adjuvant activity compared to **Natural Lipid-A**. As shown in FIG. 31 – FIG. 33, liposomal vaccine formulations containing those synthetic Lipid-A analogs **48, 54, 70,**

86, **102** and **104** induce significant T-cell blastogenesis and high levels of interferon-gamma. The values of CPM (counts per minute) and IFN-γ (pg/ml) are comparable to those observed for the formulations containing **Natural Lipid-A**. The formulations containing the synthetic Lipid-A analog **94** (FIG. 33) also induce significant T-cell blastogenesis, although the production of interferon-gamma level is low. Further testing of compound **94** is necessary to provide unambiguous result of its adjuvant properties. Compound **33**, **77** and **58** are inactive in both T-cell blastogenesis and interferon-gamma production.

Compound **86** seems to be slightly more active than all the other synthetic Lipid-A analogs tested in the second group shown in (FIG. 32). In a direct comparison with natural Lipid-A, synthetic analog **86** exhibited similar values in both T-cell blastogenesis and IFN-γ production. Structurally, compound **86** has a total of seven lipid chains, including a 3-hydroxyl myristic acid moiety on 3-O-position of the disaccharide backbone. Compound **54** and compound **70** are structurally very close to compound **86**, except that compound **54** has no lipid chain on 3-O-position and compound **70** has a simple n-propyl group on 3-O-position. Comparable results in both T-cell blastogenesis and Interferon-gamma (IFN-γ) production are obtained for compounds **54**, **70** and **86** (FIG. 32). This observation indicates that the nature of the substitute group on 3-O-position of the Lipid-A disaccharide backbone probably has little effect on the adjuvanticity of Lipid-A analogs. In addition, compounds **48** and **70** (FIG. 32), both having the same substitution pattern but differing only in lipid chain length, are almost identical in performance. This result confirms our belief that slight variations in lipid chain length do not affect much the adjuvant properties of Lipid-A analogs.

As shown in FIG. 33, compounds **102** and **104** also demonstrated similar results in comparison to both compound **86** and **Natural Lipid-A**. Interestingly, both compounds **102** and **104** contain an unnatural tri-lipid fatty acid moiety on the 2-N-position of the disaccharide backbone. In addition, unnatural ether linkage instead of the natural ester linkage, is also incorporated for the lipid-lipid connection in those multi-lipid fatty acid moieties. The finding that compound **102** and **104** display similar adjuvanticity as the **Natural Lipid-A** product further proves our idea that slight variations in the lipid portion of Lipid-A structures do not affect the adjuvant property

of these molecules. These results are extremely useful in designing new Lipid-A mimics that may provide structurally simpler, more stable, and biologically more active molecules.

Synthetic analogs **33**, **58** and **77** contain aspartic acid based lipids. The observed weak responses to these liposomal formulations are primarily attributed to their poor incorporation into liposomes as a result of their poor solubility. Different formulation techniques may be needed to assure their efficient incorporation into liposomes and to re-evaluate their adjuvant properties.

EXAMPLES

General: Melting points were not corrected. All air and moisture sensitive reactions were performed under nitrogen atmosphere. Anhydrous THF, DMF and dichloromethane were purchased from Aldrich and other dry solvents were prepared in the usual way. ACS grade solvents were purchased from Fisher and used for chromatography without distillation. TLC plates (silica gel 60 F_{254}, thickness 0.25 mm, Merck) and flash silica gel 60 (35 – 75 μm) for column chromatography were purchased from Rose Scientific, Canada. ^{1}H and ^{31}P spectra were recorded either on a Brucker AM 300 MHz or Varian Unity 500 MHz or Brucker DRX 600 MHz spectrometers with TMS as internal standard for proton chemical shifts. Optical rotations were measured on a Perkin-Elmer 241 Polarimeter at room temperature (20 - 22°C). Elemental analysis data were obtained from the Micro-analytical laboratory in the University of Alberta. Electron-spray mass spectrometric analyses were performed either on a MS50B or MSD1 SPC mass spectrometers.

Example 1 Preparation of Compound **2**

A solution of 1 (3.16 g, 7.69 mmol) in dry THF (35 ml) was cooled to –20°C. N-Methyl morpholine (0.76 ml, 0.70 g, 6.92 mmol) and isobutyl chloroformate (0.94 g, 0.90 ml, 6.92 mmol) were added. The mixture was stirred for 5 min. and nonylamine (1.27 ml, 0.99 g, 6.92 mmol) in dry THF (2 ml) was added to the above solution. The mixture was stirred at –20°C for 1 h. Methanol (3 ml) was added and the reaction mixture was concentrated in *vacuo*. The residue was purified by flash chromatography (hexane:ethyl acetate, 2:1) to give **2** (3.27 g, 79%). TLC: R_f=0.44 (hexane : ethyl acetate, 3 : 1). $[\alpha]_D^{22}$=+16.9 (c 0.64, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J=6.5 Hz, 3 H, CH_3), 1.42 (br s, 12 H, 6 CH_2), 1.50 (br s, 11 H, 3 CH_3, CH_2), 2.59 (dd, J=16.0, 6.5 Hz, 1 H, Asp-β–H, 2.93 (dd, J=16.0, 3.5 Hz, 1 H, Asp-β'-H), 3.24 (m, 2 H, NCH_2), 4.22 (t, J=7.0 Hz, 1 H, Fmoc-C*H*), 4.45 (m, 3 H, OCH_2, Asp-α-H), 5.96 (d J=7.0 Hz, 1 H, NH), 6.45 (m, 1 H, NH), 7.29 - 7.79 (m, 8 H, Ar-H). Anal. calcd for $C_{32}H_{44}N_2O_5$ (536.71): C, 71.61; H, 8.26; N, 5.22. Found: C, 71.68; H, 8.51; N, 5.27.

Example 2 Preparation of Compound **3**

Compound **2** (9.50 g, 19.47 mmol) was dissolved in dry THF (40 ml). Piperidine (10 ml) was added and the mixture stirred at room temperature for 1 h. The solvent was removed and the residue was purified by flash chromatography (hexane : ethyl acetate, 1 : 1 and then 5% methanol in dichloromethane) to give **3** (5.64 g, 92%). TLC: R_f=0.38 (5% methanol in dichloromethane), $[\alpha]_D^{22}$= -41.0 (c 0.59, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, J=6.5 Hz, CH_3), 1.22 (br s, 12 H, 6 CH_2), 1.45 (s, 9 H, 3 CH_3), 1.55 (m, 2 H, CH_2), 1.68 (s, 2 H, NH_2), 2.46 (dd, J=16.0, 8.0 Hz, 1 H, Asp-β-H), 2.83 (dd, J=16.0, 4.0 Hz, 1 H, Asp-β'-H), 3.20 (m, 2 H, NCH_2), 3.80 (dd, J=8.0, 4.0 Hz, 1 H, Asp-α-H), 7.36 (br s, 1 H, NH). Anal. calcd for $C_{17}H_{34}N_2O_3$ (314.47): C, 64.93; H, 10.90; N, 8.90. Found: C, 65.01; H, 11.20; N, 8.96.

Example 3 Preparation of Compound **4**

Tetradecanoic acid (4.51 g, 19.78 mmol) was dissolved in dry THF (50 ml). N-Methyl morpholine (3.78 ml, 3.47 g, 34.40 mmol) was added and the solution was cooled to -25°C. Isobutyl chloroformate (2.34 ml, 2.47 g, 18.06 mmol) was added dropwise and the mixture was stirred for five min. A solution of **3** (5.40 g, 17.20 mmol) in dry THF (50 ml) was added dropwise to the above solution. The mixture, after stirring at -25°C for 30 min, was allowed to warm up to room temperature within 1 h. Methanol (5 ml) was then added and the reaction mixture was stirred for five min further. It was concentrated in *vacuo* and the residue was purified by flash chromatography (dichloromethane : acetone, 30 : 1 and 25 : 1) to give **4** (7.21 g, 80%). TLC: R_f=0.37 (hexane : ethyl acetate, 3 : 1). $[\alpha]_D^{22}$= -14.4 (c 0.61, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.25 (br s, 32 H, 16 CH_2), 1.46 (br s, 11 H, 3 CH_3, CH_2), 1.54 (m, 2 H, CH_2), 2.21 (t, J=7.5 Hz, 2 H, CH_2), 2.52 (dd, J=16.5, 7.0 Hz, 1 H, Asp-β-H), 2.84 (dd, J=16.5, 4.0 Hz, 1 H, Asp-β'-H), 3.21 (m, 2 H, NCH_2), 4.71 (m, 1 H, Asp-α–H), 6.63 (t, J=5.0 Hz, 1 H, NH), 6.84 (d, J=7.0 Hz, 1 H, NH). Anal. calcd for $C_{31}H_{60}N_2O_5$ (524.83): C, 70.94; H, 11.52; N, 5.34; Found: C, 70.84; H, 11.87; N, 5.28.

Example 4 Preparation of Compound **5**

Compound **4** (7.74 g, 14.77 mmol) was dissolved in trifluoroacetic acid – water (95 : 5, v/v, 180 ml) and the solution was stirred at room temperature for 4 h. The solvent was then removed and the residue was purified by flash chromatography (2 to 4% methanol in dichloromethane) to give **5** (6.71 g, 97%). TLC: R_f=0.33 (5% methanol in chloroform). $[\alpha]_D^{22}$= -27.5 (c 0.24, chloroform).

^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.26 (br s, 32 H, 16 CH_2), 1.48 (m, 2 H, CH_2), 1.62 (m, 2 H, CH_2), 1.24 (t, J=7.5 Hz, 2 H, CH_2), 2.69 (dd, J=16.5, 7.0 Hz, 1 H, Asp-β-H), 2.89 (dd, J=16.5, 4.0 Hz, 1 H, Asp-β'-H), 3.21 (m, 2 H, NCH_2), 4.79 (m, 1 H, Asp-α-H), 6.95 (t, J=5.5 Hz, 1 H, NH), 7.05 (d, J=7.5 Hz, 1 H, NH). Anal. calcd $C_{27}H_{52}N_2O_4$ (468.72): C, 69.19; H, 11.18; N, 5.98. Found: C, 69.14; H, 11.28; N, 5.95.

Example 5 Preparation of Compound **8**

(1) Compound **6**: Zinc powder (19.6g, 299 mmol) was added to a mixture of dodecanal (25.0 g, 136 mmol.), ethyl bromoacetate (25.0 g, 150 mmol.), and 150 mL THF in presence of nitrogen atmosphere. The reaction flask was clamped into a sonication bath and the sonication was started. Iodine (3.5g, 27.2 mmol.) was added slowly into the reaction flask. After 2-3 minutes of sonication the reaction was initiated vigorously. Then the sonication was stopped and the reaction flask was removed from the sonicator bath. Zinc was filtered off and washed with THF (10 mL) for 6 - 7 times. The combined filtrates were concentrated in *vacuo*. A pale yellow viscous liquid was resulted to which hexane (200 mL) was added and shaken well for 5 min. A white solid precipitated out, which was filtered off and washed with hexane (3 x 15 mL). The combined filtrate and washings were concentrated to dryness and the residue was purified by column chromatography (10% ethyl acetate in hexane) to afford the pure compound **6** (22.2 g, 60%). TLC: R_f=0.25 (10% ethyl acetate in hexane). ^1H NMR (300 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, CH_3), 1.26 (m, 16 H), 1.28 (t, J=6.5 Hz, 3 H, CH_3), 1.40 – 1.50 (m, 4 H), 2.40 (dd, J=16.0, 7.0 Hz, 1 H), 2.50 (dd, J=16.0, 3.0 Hz, 1 H), 3.05 (s, 1 H, OH), 4.00 (m, 1 H, H-3), 4.17 (q, J=6.5 Hz, 2 H).

(2) Compound **7**: 3-Hydroxy-myristic ester **6** (122.0 g, 0.5 mmol) was dissolved in ethanol (800 ml) and KOH (33.6 g) was added. The reaction mixture was refluxed for 1 h and then 10% HCl (1000 ml) was added after cooling in an ice bath. A white solid precipitated out which was filtered off and dried briefly (192.0 g). The solid was recrystalized with boiling hexane (800 ml) to give a white crystalline compound **7** (98.2 g, 90%). TLC: R_f=0.35 (5% methanol in dichloromethane). ^1H NMR (300 MHz, DMSO-d_6): δ=0.88 (t, J=6.5 Hz, CH_3), 1.25 (m, 20 H), 2.20 (dd, J=15.0, 7.5 Hz, 1 H), 2.28 (dd, J=15.0, 5.5 Hz, 1 H), 3.80 (br s, 1 H), 4.55 (br s, 1 H).

(3) **3-hydroxy-myristic acid – dehydroabietyl amine salt**: Pure dehydroabietyl amine (purified according to lit.[21b], 131.0 g, 459.6 mmol) was dissolved in a mixture of hexane (3.0 L)

and diethyl ether (1.5 L). Compound **7** (110.0 g, 450.8 mmol) was dissolved in diethyl ether (3.0 L) and added to the above amine solution with stirring. Crystallization started immediately after the addition. The stirring was stopped and the reaction mixture left at room temperature for 1 h. The white solid was filtered out and washed with hexane : diethyl ether mixture (1 : 1). The precipitate was dissolved in methanol (500 ml) (heated to dissolve) and then hexane : diethyl ether mixture (1 : 1, 700 ml) was added. The mixture was kept in the refrigerator (- 9°C) overnight. A white solid precipitated out which was collected by filtration. The recystalization of the solid was repeated until there was no further rise in melting point. The final yield of the pure (R)- salt was 38 g, 40%. m.p. 131.5 – 132°C. ^{1}H NMR (600 MHz, CDCl3) δ: 0.85 (t, J=6.5 Hz, 3 H, CH_3), 1.00 (s, 3 H, CH_3), 1.19 (d, J=6.5 Hz, 6 H, 2 CH_3), 1.25 (s, 3 H, CH_3), 1.25 – 1.70 (m), 2.10 (dd, J=15.0, 10.0 Hz, 1 H), 2.27 (m, 2 H), 2.63 (d, J=12.5 Hz, 1 H), 2.79 (m, 2 H), 2.87 (m, 2 H), 3.81 (m, 1 H), 6.40 (br s, 3 H), 6.85 (d, J=1.5 Hz, 1 H), 6.95 (dd, J=8.0, 1.5 Hz, 1 H), 7.12 (d, J=8.0 Hz, 1 H). A single set of double doublet peaks at 2.10 ppm is characteristic of only one isomer present. For the salt formed from racemic 3-hydroxy myristic acid, two sets of double doublet peaks will appear at this position. NMR can be used here to detect the separation efficiency.

(4) Compound **8**: The salt of dehydroabietyl amine and myristic acid (38.0g) was taken into a 2 L round bottomed flask and saturated aqueous sodium carbonate (1000 ml) and diethyl ether (800 ml) were added. The mixture was stirred vigorously for 30 min. The water layer and ether layer were separated and the solid was collected. The solid was washed with water (300 ml), ether (3x100 ml) and dried briefly. The solid was treated with 1 L of 2% HCl (aq.) and ethyl acetate (1.5 L) till the solid dissolved completely. The aqueous layer was separated and extracted with ethyl acetate (2x150 ml). The organic layer was dried with anhydrous sodium sulfate and concentrated to give R-rich acid that was recrystalized from ethyl acetate and hexane to provide **8** (16.5 g) in ~95% e.e. $[\alpha]_D^{20}$= - 15.5 (c 1.0, $CHCl_3$).

Example 6 Preparation of Compound **9**

Compound **8** (16.0g, 65.6 mmol) was dissolved in anhydrous ethyl acetate and bromoacetophenone (15.66g, 78.7 mmol) and triethyl amine (7.95g, 78.7 mmol) were added under N_2 atmosphere. The reaction mixture was allowed to stir overnight. A white solid was precipitated out which was filtered off and washed with ethyl acetate (3 X 10 ml). The combined

washings and filtrate was evaporated to get a pale yellow solid which was purified to give **9** (22.0g, 92%) as white crystalline solid by silica gel chromatography (ethyl acetate : hexane, 1 : 8). TLC: R_f=0.42 (ethyl acetate : hexane, 1:3). $[\alpha]_D^{20}$=-5.7 (c 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 3 H, CH_3), 1.30 (br s, 16 H), 1.40 – 1.60 (m, 4 H), 2.57 (dd, J=15.0, 9.0 Hz, 1 H), 2.70 (dd, J=15.0, 3.0 Hz, 1 H), 3.45 (d, J=3.5 Hz, 1 H), 4.18 (m, 1 H, H-3), 5.37 (d, J=16.0 Hz, 1 H), 5.49 (d, J=16.0 Hz, 1 H), 7.50 (m, 2 H), 7.64 (m, 1 H), 7.92 (m, 2 H).

Example 7 Preparation of Compound **10**

Compound **9** (500 mg, 1.381 mmol) was dissolved in pyridine (5 ml) and to this solution 4-dimethylaminopyridine (DMAP, 8.46 mg, 0.068 mmol) was added. The resulting mixture was then cooled in an ice bath for 5 minutes before lauroyl chloride (383 μl, 1.657 mmol) was added dropwise. When the addition of lauroyl chloride ended, the entire mixture was left with stirring at room temperature overnight. TLC of reaction mixture in the next morning showed the presence of starting lipid **9**. The reaction was then cooled down to 0°C and at this temperature it was treated with some more lauroyl chloride (160 μl). Subsequently, the reaction mixture was removed from the bath and left to react for 3 hours further at room temperature. To stop the reaction, methanol (1 ml) was added and 10 minutes later reaction mixture was concentrated to dryness by evaporator. The residue was then applied on silica gel for chromatographic purification (ethyl acetate : hexane; 1 : 9) to provide compound **10** (524 mg, 70%). Yield of reaction could be higher if the impure fraction (320 mg) was purified again. TLC: R_f=0.34 (ethyl acetate : hexane, 1:9). $[\alpha]_D^{20}$= +1.0 (c 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.30 (m, 34 H), 1.65 (m, 4 H), 2.30 (t, J=6.5 Hz, 2 H), 2.72 (dd, J=15.5, 5.5 Hz, 1 H), 2.78 (dd, J=15.5, 7.5 Hz, 1 H), 5.30 (m, 1 H, H-3), 5.35 (s, 2 H), 7.47 (m, 2 H), 7.63 (m, 1 H), 7.90 (m, 2 H).

Example 8 Preparation of Compound **11**

Compound **9** (16.0 g, 44.2 mmol) was dissolved in pyridine (150 ml) and DMAP (270 mg, 2.2 mmol) was added. Tetradecanoyl chloride (13.1 g, 53.0 mmol) was added dropwise while keeping the reaction mixture in cold water bath. The reaction mixture was stirred for 3 h at room temperature. When TLC indicated the completion of the reaction, methanol (20 ml) was added to quench the reaction and allowed to stir for 30 min. The solvents were removed to dryness and the

residue was dissolved in 800 ml ethyl acetate and washed with ice water (2 x 100 ml). The aqueous layer was back extracted with ethyl acetate (3 x 100 ml). The combined organic layer was dried over anhydrous sodium sulphate and the solvent was removed in *vacuo*. The colourless residue obtained was purified on a silica gel chromatography (ethyl acetate : hexane, 1 : 10) to give the pure compound **11** (23.0g, 92%). TLC: R_f=0.54 (ethyl acetate : hexane, 5 : 1). $[\alpha]_D^{20}$=+0.95 (c 2.0, chloroform). ^1H NMR (300 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 6 H, 2 CH3), 1.26 (br s, 38 H), 1.65 (m, 4 H), 2.31 (t, J=6.5 H, 2 H), 2.72 (dd, J=16.0, 5.5 Hz, 1 H), 2.79 (dd, J=16.0, 7.5 Hz, 1 H), 5.32 (m, 1 H, H-3), 5.34 (s, 2 H), 7.35 (m, 2 H), 7.61 (m, 1 H), 7.90 (m, 2 H).

Example 9 Preparation of Compound **12**

Compound **12** was synthesized by following exactly the same procedure described for the preparation of compound **10**, except that lauroyl chloride was replaced by palmitoyl chloride. When the reaction between compound 9 (500 mg, 1.381 mmol) and palmitoyl chloride (628.5 μl) was completed, the residue from solvent removal was re-dissolved in ethyl acetate and washed with water. The organic layer was dried over Na_2SO_4 and concentrated. Desired product, compound 12, was obtained as colourless solid (463.4 mg, 56%) from silica gel column purification using ethyl acetate-hexane (1 : 9) for elution. Additional desired product (563.6 mg) was obtained in slightly impure form. TLC: R_f $[\alpha]_D^{20}$= +2.0 (c 1.0, chloroform). ^1H NMR (300 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.30 (br s, 42 H), 1.65 (m, 4 H), 2.31 (t, J=6.5 Hz, 2 H), 2 72 (dd, J=15.0, 5.5 Hz, 1 H), 2.78 (dd, J=15.0, 7.5 Hz, 1 H), 5.30 (m, 1 H, H-3), 5.35 (s, 2 H), 7.48 (m, 2 H), 7.61 (m, 1 H), 7.90 (m, 2 H).

Example 10 Preparation of Compound **13**

Compound **10** (510 mg) was dissolved in 80% acetic acid in ethyl acetate (18 ml) and Zn powder (1.0 g) was added. The entire mixture was left with good stirring overnight. Next morning the reaction was treated with additional Zn powder (400 mg) and stirred for 5 hours further. The solid was then filtered off and washed with generous amount of ethyl acetate. The filtrate was concentrated to dryness. The remainder was purified on flash silica column (ethyl acetate : hexane : acetic acid , 1 : 10 : 1%) to yield compound **13** (327 mg, 82%) as colourless solid on cooling at 0°C. TLC: R_f=0.22 (ethyl acetate : hexane, 1 : 9). $[\alpha]_D^{20}$= - 1.2 (c 1.0, chloroform). ^1H

NMR (300 MHz, $CDCl_3 - CD_3OD$): δ=0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.30 (br s, 34 H), 1.62 (m, 4 H), 2.30 (t, J=6.5 Hz, 2 H), 2.52 (dd, J=15.5, 5.5 Hz, 1 H), 2.60 (dd, J=15.5, 7.5 Hz, 1 H), 5.22 (m, 1 H, H-3).

Example 11 Preparation of Compound **14**

The mixture of compound **11** (23.0 g), zinc powder (50 g) and glacial acetic acid (350 ml) was stirred at room temperature for 2 h. Zinc powder was filtered off and washed with ethyl acetate (100 ml). Combined filtrates were concentrated under reduced pressure and co-evaporated with toluene twice. Residue as colourless viscous liquid was purified by silica gel chromatography (2% methanol in dichloromethane). The pure compound **14** (16.5 g, 90%) was obtained as a white solid after evaporation of the solvent. TLC: R_f=0.40 (ethyl acetate : hexane, 1 : 3). $[\alpha]_D^{20}$= - 1.25 (c 2.0, $CHCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.30 (br s, 38 H), 1.40 – 1.55 (m, 4 H), 2.47 (dd, J=16.5, 9.0 Hz, 1 H), 2.58 (dd, J=16.5, 3.0 Hz, 1 H), 4.05 (m, 1 H, H-3).

Example 12 Preparation of Compound **15**

Compound **12** (429.9 mg, 0.716 mmol) was dissolved in ethyl acetate (4.4 ml) and acetic acid (17.6 ml) was added. After an addition of Zn powder (2.1 g) was made, the mixture was stirred at room temperature overnight. Zn was then removed by filtration and washed with generous amount of ethyl acetate. The filtrate was concentrated and purified on flash silica gel column (ethyl acetate : hexane : acetic acid, 1 : 10 : 1 %) to give compound **15** (245 mg, 72 %). TLC: R_f =0.25 (ethyl acetate : hexane, 1 : 10). $[\alpha]_D^{20}$= -1.3 (c 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.23 (br s, 42 H), 1.62 (m, 4 H), 2.28 (t, J=6.5 Hz, 2 H), 2.56 (dd, J=16.0, 5.5 Hz, 1 H), 2.64 (dd, J=16.0, 7.5 Hz, 1 H), 5.20 (m, 1 H, H-3).

Example 13 Preparation of Compound **16**

Compound 9 (5.0 g, 13.8 mmol), benzyl trichloroacetimidate (7.0 g, 27.6 mmol), molecular sieve powder (4 Å, 3.0 g), anhydrous dichloromethane (100 ml) and anhydrous hexane (100 ml) were taken in a 250 ml round bottomed flask and stirred for 30 min under N_2 atmosphere. Cooled to 0°C and trifluoromethyl sulfonic acid (0.414 g, 2.8 mmol) was added and stirred for 5 h at 0°C.

When there was no further improvement in the formation of product shown on TLC, the reaction was quenched with triethyl amine (2 ml). The reaction mixture was filtered through a small celite bed, washed thoroughly with dichloromethane and the combined filtrate and washings were concentrated. The colorless residue obtained was purified on a silica column (ethyl acetate : hexane, 1 : 10) to obtain pure **16** (6.0 g, 96%) as a shiny white solid. TLC: R_f=0.36 (hexane : ethyl acetate, 6 : 1). $[\alpha]_D^{20}$= -7.4 (c 2.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 3 H, CH_3), 1.25 (br s, 18 H), 1.62 (m, 2 H), 2.66 (dd, J=15.0, 5.5 Hz, 1 H), 2.80 (dd, J=15.0, 7.5 Hz, 1 H), 3.95 (m, 1 H), 4.54 (d, J=11.5 Hz, 1 H), 4.60 (d, J=11.5 Hz, 1 H), 5.27 (d, J=15.5 Hz, 1 H), 5.35 (d, J=15.5 Hz, 1 H), 7.32 (m, 5 H, Ar-H), 7.47 (m, 2 H), 7.60 (m, 1 H), 7.90 (m, 2 H).

Example 14 Preparation of Compound **17**

By following the same procedure described for the preparation of compound **14**, compound **17** was prepared from compound **16** (6.0 g) in the presence of zinc powder (10.0 g) and glacial acetic acid (100 ml). Reaction was completed in 30 min. After the work up, the colourless residue was purified on a silica column with ethyl acetate: hexane : acetic acid (2 : 8 : 1%) as solvent system. Evaporation of the solvent afforded **17** as a white solid in 65% yield. TLC: R_f=0.45 (hexane : ethyl acetate, 4 : 1). $[\alpha]_D^{20}$= - 5.4 (c 2.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 3 H, CH_3), 1.26 (br s, 18 H), 1.60 (m, 2 H), 2.55 (dd, J=15.5, 5.5 Hz, 1 H), 2.64 (dd, J=15.5, 6.5 Hz, 1 H), 3.87 (m, 1 H), 4.58 (s, 2 H), 7.30 (m, 5 H, Ar-H).

Example 15 Preparation of Compound **19**

(1) Compound **18**: A mixture of dodecyl alcohol (810.0 mg, 4.348 mmol), pyridine (394 μl) and CH_2Cl_2 (0.5 ml) was slowly added to a cooled solution of trifluoromethyl sulfonic acid anhydride (triflic anhydride, 1.433 ml in 8.0 ml of CH_2Cl_2) at 0°C over 15 minutes period. The resulting mixture was then stirred at the same temperature for 20 minutes at which time the reaction appeared as slightly orange suspension and its TLC indicated the absence of dodecyl alcohol. Cooled hexane (20 ml) was added. Solid was then removed by filtering through a Na_2SO_4 bed and washed with some more hexane. The removal of solvent provided crude triflate product as clear liquid. After drying further under high vacuum the amount of crude triflate **18** was 1.433g. TLC: R_f=0.77 (hexane : ethyl acetate, 10 : 1).

(2) Compound **19**: Lipid **9** (1.05 g; 2.989 mmol) and Na_2SO_4 (7.0 g) in dry 1,2-dichoroethane (25 ml) was stirred for 5 minutes before crude triflate **18** solution (1.433g in 2.0 ml of dry 1,2-dichoroethane) was added. The resulting suspension was stirred at room temperature over weekend. It was then heated at 80°C for 2 hours. Still some lipid **9** remained in the reaction mixture, but the reaction was terminated. Solid was filtered through a Na_2SO_4 bed and washed with CH_2Cl_2 (150 ml). Combined filtrates were washed with saturated $NaHCO_3$ (20 ml), water (50 ml), and dried over Na_2SO_4. Remainders from solvent removal were purified on flash silica gel column (CH_2Cl_2 : hexane, 1 : 1) to give slightly impure **19** which was re-purified on a second column (CH_2Cl_2 : hexane, 4 : 1) to render pure **19** (1.05 g, 69 %). TLC: R_f=0.29 (hexane / ethyl acetate, 10 :1). $[\alpha]_D^{20}$= - 3.8 (c 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.26 (br s, 36 H), 1.55 (m, 4 H), 2.60 (dd, J=15.5, 5.5 Hz, 1 H), 2.74 (dd, J=15.5, 7.5 Hz, 1 H), 3.46 (m, 2 H), 3.76 (m, 1 H), 5.30 (d, J=16.5 Hz, 1 H), 5.39 (d, J=16.5 Hz, 1 H), 7.49 (m, 1 H), 7.61 (m, 1 H), 7.91 (m, 2 H).

Example 16 Preparation of Compound **20**

Using the same procedure described for the synthesis of compound **14**, compound **20** was prepared from the reaction of compound **19** (1.02 g; 1.922 mmol) with Zn powder (1.12 g) in acetic acid (12.0 ml). After stirring at room temperature overnight, Zn was removed and combined filtrates were evaporated to dryness. Residue was applied on flash silica gel column for purification, initially with CH_2Cl_2 : hexane (8 : 1) to remove all impurities with high R_f value and later ethyl acetate : hexane (1 : 5) to elute desired produce **20** in 88% yield (741.5 mg). TLC: R_f=0.20 (dichloromethane : hexane, 8 : 1). $[\alpha]_D^{20}$= - 4.8 (c 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.27 (br s, 36 H), 1.56 (m, 4 H), 2.54 (d, J=6.0 Hz, 2 H), 3.50 (m, 2 H), 3.69 (m, 1 H).

Example 17 Preparation of Compound **21**

(-)-DIP-Cl (9.6 g, 30 mmol) was dissolved in anhydrous diethyl ether (35 ml, freshly distilled over Na/benzophenone) under nitrogen atmosphere. The mixture was cooled to –40°C (dry ice / acetone). Allyl magnesium bromide (Aldrich, 1.0 M solution in diethyl ether was considered as 0.9 M solution and hence 27.7 ml, 25 mmol) was added slowly to the above cooled solution. The reaction mixture was allowed to warm to room temperature and stirring was continued for 30

min (total stirred for 1.0 h). Meanwhile dodecanal (4.41 ml, 3.68 g, 20 mmol) was dissolved in dry diethyl ether and cooled to 0°C using an ice bath.

The above reaction mixture which was stirred at room temperature for 30 min was cooled to –78°C and the cooled dodecanal solution was added slowly within 15 min and the stirring was continued at –78°C for 1.0 h. When TLC (ethyl acetate : hexane, 1 : 15) showed almost completion of the reaction, the cold bath was removed and the reaction mixture allowed to stir for 5-10 min. Saturated aqueous sodium acetate solution (5.0 ml) and hydrogen peroxide (50%, 5.0 ml) were added slowly and cautiously and stirred for 5 min. The addition of sodium acetate solution and hydrogen peroxide was repeated at every 5 min. intervals of time, total 20 ml of sodium acetate and 15 ml of hydrogen peroxide were added. During the addition of these reagents, the reaction mixture become warmer and care should be taken that the temperature does not rise too high (if necessary use ice bath to cool). The resultant mixture was extracted with diethyl ether (3 X 20 ml). The combined ether extracts were washed with water and brine (1 X 25 ml) and dried over anhydrous sodium sulfate (60 g) and concentrated to obtain a colorless oily compound. Purification by silica gel column chromatography using 10% hexane in ethyl acetate afforded pure **21** as colorless viscous oil in 60% yield. TLC: R_f=0.26 (hexane / ethyl acetate, 15 : 1). $[\alpha]_D^{20}$=+4.9 (c 0.57, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 3 H, CH_3), 1.28 (br s, 18 H), 1.46 (m, 2 H), 2.09 –2.18 (m, 1 H), 2.30 (m, 1 H), 3.63 (m, 1 H, OH), 5.13 (m, 2 H), 5.83 (m, 1 H).

Example 18 Preparation of Compound **22**

Compound **20** (250 mg, 0.61 mmol) and **21** (272 mg, 1.21 mmol) were dissolved in dry dichloromethane (5 ml). DCC (377 mg, 1.83 mmol) and DMAP (15.0 mg, 0.12 mmol) were added and the mixture was stirred at room temperature for 24 h. The reaction was quenched by adding water (0.3 ml) and methanol (5 ml) and the mixture stirred for 10 min. The solution was concentrated to dryness in *vacuo* by codistillation with chloroform (10 ml) twice and the residue treated with hexane (10 ml). The solid was filtered out and the filtrate concentrated in *vacuo*. The residue was purified by flash silica gel chromatography (hexane / ethyl acetate, 30 : 1) to give **22** (302 mg, 81%). TLC: R_f=0.33 (hexane / ethyl acetate, 3 : 1). $[\alpha]_D^{20}$=+8.2 (c 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.89 (t, J=6.5 Hz, 9 H, 3 CH_3), 1.26 (br s, 54 H). 1.52 (m, 6 H),

2.31 (m, 2 H), 2.36 (dd, J=15.5, 6.5 Hz, 1 H), 3.43 (m, 2 H), 3.68 (m, 1 H), 4.93 (m, 1 H), 5.07 (m, 2 H), 5.75 (m, 1 H). ES-MS calcd for $C_{41}H_{80}O_3$: 620.6; Found: 621.9 ($M+H^+$).

Example 19 Preparation of Compound **23**

Compound **22** (280 mg, 0.45 mmol) was dissolved in hexane (10 ml) and acetic acid (2 ml) and Aliquat (3 drops) was added. The mixture was cooled to 0°C and potassium permanganate solution (980 mg in 15 ml water) was added. The reaction mixture was stirred at 0°C for 6 h. When the reaction was complete, sodium sulfite (2.5 g) and hydrogen chloride solution (6 N, 5 ml) were added. The dark brown solid disappeared and the mixture became a clear solution. The mixture was extracted with hexane (30 ml x 3) and with dichloromethane (30 ml x 3). Both hexane and dichloromethane extracts contained product and they were washed, separately, with saturated sodium chloride solution (20 ml), dried with sodium sulfate and concentrated in *vacuo*. The combined residue was purified by flash silica gel chromatography (ethyl acetate : hexane, 1 : 3) to give **23** (176 mg, 61%). TLC: R_f=0.30 (ethyl acetate : hexane, 1 : 3). $[\alpha]_D^{20}$= -2.4 (c 0.5, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.89 (t, J=6.5 Hz, 9 H, 3 CH_3), 1.27 (br s, 54 H). 1.48 – 1.65 (m, 6 H), 2.38 (dd, J=15.0, 6.0 Hz, 1 H), 2.53 (dd, J=15.0, 7.0 Hz, 1 H), 2.57 (dd, J=15.5, 5.5 Hz, 1 H), 2.66 (dd, J=15.5, 7.0 Hz, 1 H), 3.42 (m, 2 H), 3.67 (m, 1 H), 5.22 (m, 1 H). ES-MS calcd for $C_{40}H_{78}O_5$: 638.6; Found: 639.3 ($M+H^+$).

Example 20 Preparation of Compound **26**

(1) D-glucosamine hydrogen chloride (195 g, 0.88 mmol) and sodium bicarbonate (152 g, 1.89 mmol) were dissolved in water (2 L) in plastic container with mechanic stirring. To this mixture trichloroethoxy carbonyl chloride (180 g, 0.85 mmol) was slowly added within 35 min. After the end of addition, stirring was continued for 1.5 h. The reaction mixture was then acidified by 10% HCl solution (300 ml). The solid was filtered off and washed with water (800 ml) and diethyl ether (500 ml), dried at 50°C under *vacuo* to give the N-Troc-protected D-glucosamine (275 g, 91%) which was used directly for the next step reaction.

(2) Compound **24**: Hydrochloric acid (g) was bubbled into benzyl alcohol (30 ml, 282 mmol) for 12 min at 0°C to provide about 1.45 g of HCl. This benzyl alcohol and acid solution was then added to an around bottom flask containing N-Troc-protected D-glucosamine (10.0 g, 28.2 mmol). The mixture as suspension was heated at 100°C for 25 min. and upon the heating, the

suspension gradually became a clear solution. After cooling the reaction at room temperature for 30 min. it was treated with hexane (200 ml) and vigorously swirled. Discarded the upper organic layer and kept the milky solid remained at the bottom of the flask. Repeated this treatment twice on the milky solid with different solvents [(ethyl acetate : hexane (1 : 4, 50 ml) and ethyl acetate (50 ml)]. Further treatment of the milky solid with hexane (200 ml) gave **24** (8.72 g, 70%) as crude product which was free from benzyl alcohol and used in the next step reaction.

(3) Compound 26: Compound **24** (3.14 g, 7.06 mmol) was suspended in dry acetonitrile (50 ml). Benzaldehyde dimethyl acetal (3.21 ml, 21.38 mmol) and p-toluene sulfonic acid (50 mg, 0.26 mmol) were added. The mixture was stirred at room temperature for 4 h, and triethyl amine (1 ml) was added to quench the reaction. The solvent was then removed and the residue was purified through recrystalization from ethyl acetate and hexane to give **26** (3.63 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.52 (s, 1 H, OH), 3.59 (m, 1 H), 3.77 (dd, J=10.0, 10.0 Hz, 1 H), 3.85 – 4.00 (m, 3 H), 4.25 (dd, J=11.0, 5.5 Hz, 1 H), 4.55 (d, J=12.0 Hz, 1 H), 4.60 – 4.84 (m, 3 H), 4.99 (d, J=3.5 Hz, 1 H, H-1), 5.30 (d, J=10.0 Hz, 1 H, NH), 5.57 (s, 1 H), 7.25 – 7.50 (m, 10 H, Ar-H).

Example 21 Preparation of Compound **27**

A mixture of crude **24** (1.35 g, 3.04 mmol) and anhydrous $CaSO_4$ (0.6 g) in anhydrous CH_2Cl_2 (16 ml) was stirred at room temperature for 5 minutes. To this suspension, 2,2-dimethoxy propane (1.2 ml, 9.12 mmol) was added and followed by an addition of p-toluene sulfonic acid monohydrate (62 mg, 0.30 mmol). The resulting mixture was then allowed to react for 1.5 hours. The reaction mixture was then neutralized with solid $NaHCO_3$ and solid was then filtered off and washed with CH_2Cl_2. Residue from solvent and reagent removal was purified on flash silica gel column (ethyl acetate : hexane; 1 : 2) to give compound **27** as colorless foam (1.19 g, 81%). TLC: R_f=0.67 (hexane : ethyl acetate, 3 : 2). $[\alpha]_D^{20}$= + 104.2 (c 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.45 (s, 3 H, CH_3), 1.55 (s, 3 H, CH_3), 3.59 – 3.88 (m, 5 H), 3.93 (ddd, J=9.5, 9.5, 3.5 Hz, 1 H, H-2), 4.49 (d, J=11.5 Hz, 1 H), 4.65 (d, J=12.0 Hz, 1 H), 4.72 (d, J=11.5 Hz, 1 H), 4.80 (d, J=12.0 Hz, 1 H), 4.93 (d, J=3.5 Hz, 1 H, H-1), 5.32 (d, J=9.5 Hz, 1 H, NH), 7.20 (m, 5 H, Ar-H).

Example 22 Preparation of Compound **28**

(1) Compound **25**: Hydrochloric acid (g) was bubbled into allyl alcohol (190 ml) at 0°C for about 30 minutes to provide approximate 9.5 g of HCl. This allyl alcohol and HCl solution was transferred into a round bottom flask containing N-Troc-protected-D-glucosamine (see Example 20 / (1), 69 g; 194.58 mmol). The whole mixture appeared as suspension and it became clear solution after 5 minutes immersion in 104°C bath. Heating was continued for 35 minutes at the same temperature. After removing the reaction mixture from the bath and let it cooled to room temperature, allyl alcohol was then removed by evaporator. Co-evaporation with toluene was also performed to remove any moisture to provide crude **25** as brownish flakes, which was used directly in the next step reaction. TLC: R_f=0.64 (10 % methanol in dichloromethane).

(2) Compound **28**: The above crude **25** was then dissolved in CH_3CN (600 ml) and treated with benzaldehyde dimethyl acetate (87 ml) and p-toluene sulfonic acid monohydrate (490 mg, 2.40 mmol) at room temperature for 6.5 hours. Reaction mixture was then treated with solid $NaHCO_3$ (12 g) to obtain the alkaline pH. Solid was filtered off and washed with acetone and combined filtrates were concentrated to dryness. Desired product **28**, as colourless solid (45.74 g ,49%), was obtained from ethanol (275 ml) recrystalization. TLC: R_f=0.22 (hexane / ethyl acetate, 3 : 1). $[\alpha]_D^{20}$= + 69.3 (c 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.50 (br s, 1 H, OH), 3.56 (dd, J=9.5, 9.5 Hz, 1 H), 3.75 (dd, J=10.0, 10.0 Hz, 1 H), 3.82 – 4.04 (m, 4 H), 4.18 – 4.30 (m, 2 H), 4.68 (d, J=11.5 Hz, 1 H), 4.82 (d, J=11.5 Hz, 1 H), 4.92 (d, J=3.5 Hz, 1 H, H-1), 5.25 (d, J=10.0 Hz, 1 H, NH), 5.28 – 5.35 (m, 2 H), 5.55 (s, 1 H), 5.89 (m, 1 H), 7.38 (m, 3 H), 7.50 (m, 2 H).

Example 23 Preparation of Compound **29**

Tetradecanoic acid (1.29 g, 5.55 mmol) and **26** (2.00 g, 3.76 mmol) were dissolved in dry dichloromethane (50 ml) under nitrogen. To the solution were added dicyclohexyl carbodiimide (DCC, 1.17 g, 5.66 mmol) and 4-dimethylaminopyridine (DMAP, 0.23 g, 1.89 mmol). The mixture was stirred at room temperature for 2 h and the solid was filtered and washed with dichloromethane (4 ml). The filtrate was concentrated in *vacuo* and the residue purified by silica gel chromatography (hexane : ethyl acetate, 6 : 1) to give **29** (2.53 g, 91%). TLC: R_f=0.40 (hexane : ethyl acetate, 6 : 1). $[\alpha]_D^{22}$=+44.7 (c 0.57, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J=6.5 Hz, 3 H, CH_3). 1.25 (m, 20 H), 1.57 (m, 2 H, CH_2), 2.30 (m, 2 H, CH_2), 3.72 (dd, J=10.0, 10.0 Hz, 1 H, H-4), 3.79 (dd, J=10.0, 10.0 Hz, 1 H, H-6), 3.97 (ddd, J=10.0, 10.0, 5.5 Hz,

1 H, H-5), 4.05 (ddd, J=10.0, 10.0, 3.5 Hz, 1 H, H-2), 4.21 (dd, J=10.0, 5.5 Hz, 1 H, H-6'), 4.54 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.66, 4.71 (2d, J=12.0 Hz, each 1 H, Cl_3CCH_2O), 4.76 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.97 (d, J=3.5 Hz, 1 H, H-1), 5.35 (d, J=10.0 Hz, 1 H, NH), 5.41 (dd, J=10.0, 10.0 Hz, 1 H, H-3), 5.53 (s, 1 H, C*H*Ph), 7.30 – 7.45 (m, 10 H, Ar-H). Anal. calcd for $C_{37}H_{50}Cl_3NO_8$ (743.16): C, 59.80; H, 6.78; N, 1.88. Found: C, 60.03; H, 6.63; N, 1.97.

Example 24 Preparation of Compound **30**

Compound **29** (2.49 g, 3.35 mmol) was converted to **30** by activated Zinc (6.56 g, 100.9 mmol) and 80% acetic acid in ethyl acetate (150 ml) as its described for the preparation of **13**. Reaction was completed in 6 hours and with a usual work up residue was obtained. It was then lyophilized from dioxane to give the free amine compound (1.83 g, 96%). TLC: R_f=0.41 (5% methanol in dichloromethane).

The above amino compound (900 mg, 1.59 mmol) was re-dissolved in dry CH_2Cl_2-DMF (4 : 1, 100 ml). To this amine solution and under nitrogen atmosphere, compound **5** (1.15 g, 2.46 mmol) and DCC (528 mg, 2.46 mmol) were added. The reaction mixture was stirred at room temperature for 48 h. The removal of solvent gave the residue which was purified by repeated flash chromatography (initially with 3 to 5% acetone in chloroform and subsequently with 8 to 10% acetonitrile in dichloromethane) to give **30** (1.08 g, 62%). TLC: R_f=0.42 (5% methanol in dichloromethane). $[\alpha]_D^{22}$=+31,0 (c 0.29, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, J=6.5 Hz, 9 H, 3 CH_3), 1.23 (m, 52 H, 26 CH_2), 1.45 – 1.65 (m, 6 H, 3 CH_2), 2.18 – 2.35 (m, 5 H, 2 CH_2, Asp-β-H), 2.70 (dd, J=15.0, 3.5 Hz, 1 H, Asp-β'-H), 3.16 (m, 2 H, NCH_2), 3.68 (dd, J=10.0, 10.0 Hz, 1 H, H-4), 3.75 (dd, J=10.0, 10.0 Hz, 1 H, H-6), 3.93 (ddd, J=10.0, 10.0, 3.5 Hz, 1 H, H-5), 4.20 (dd, J=10.0, 3.5 Hz, 1 H, H-6'), 4.30 (ddd, J=10.0, 10.0, 3.5 Hz, 1 H, H-2), 4.58, 4.70 (2 d, J=11.0 Hz, each 1 H, C*H₂*Ph), 4.60 (m, 1 H, Asp-α-H), 4.97 (d, J=3.5 Hz, 1 H, H-1), 5.32 (dd, J=10.0, 10.0 Hz, 1 H, H-3), 5.50 (s, 1 H, C*H*Ph), 6.13 (d, J=10.0 Hz, 1 H, NH), 6.97 (t, J=5.0 Hz, 1 H, NH), 7.25 – 7.44 (m, 11 H, NH, Ar-H). ES-MS calcd for $C_{61}H_{99}N_3O_9$: 1017.7; Found: 1019.3 (M+H).

Example 25 Preparation of Compound **31**

To a solution of **30** (150 mg, 0.15 mmol) in dry THF - $CHCl_3$ (6 : 1, 24 ml), molecular sieves (4A, 0.5 g) were added and the mixture was stirred at room temperature for 20 minutes. Sodium

cyanoborohydride (188 mg, 3.00 mmol) was added and the mixture was cooled to 0°C where the HCl(g) / Et_2O was added dropwise slowly till no gas was evolved. Additional sodium cyanoborohydride (400 mg) was added, followed by slow addition of HCl (g) / Et_2O until no gas was formed. The mixture was poured into saturated sodium bicarbonate solution (50 ml) and extracted with ethyl acetate (100 ml x 3). The organic layer was washed with saturated sodium chloride solution (15 ml), dried over sodium sulfate, concentrated. The residue was purified by flash chromatography (1 to 2% methanol in dichloromethane) to give **31** (130 mg, 86%). TLC: R_f=0.46 (dichloromethane : methanol, 95 : 3, developed for two times). $[\alpha]_D^{22}$= +30.7 (c 0.46, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J=6.5 Hz, 9 H, 3 CH_3), 1.25 (br. s, 52 H, 26 CH_2), 1.45 (m, 2 H, CH_2), 1.60 (m, 4 H, 2 CH_2), 2.21 (m, 2 H, CH_2), 2.30 (m, 2 H, CH_2), 2.35 (dd, J=15.0, 7.0 Hz, 1 H, Asp-β-H), 2.71 (dd, J=15.0, 3.5 Hz, 1 H, Asp-β'-H), 2.72 (d, J=3.0 Hz, 1 H, OH), 3.17 (m, 2 H, NCH_2), 3.65 – 3.87 (m, 4 H, H-4, H-5, 2 H-6), 4.25 (ddd, J=10.5, 9.5, 3.5 Hz, 1 H, H-2), 4.54 – 4.73 (m, 5 H, 2 CH_2Ph, Asp-α-H), 4.96 (d, J=3.5 Hz, 1 H, H-1), 5.13 (dd, J=10.5, 9.0 Hz, 1 H, H-3), 6.13 (d, J=9.0 Hz, 1 H, NH), 7.02 (t, J=5.5 Hz, 1 H, NH), 7.28 – 7.40 (m, 11 H, NH, Ar-H). Anal. calcd for $C_{61}H_{101}N_3O_9$: C, 71,80; H, 9.98 N, 4.12. Found: C, 71.66; H, 10.39; N, 4.48. ES-MS calcd 1019.8; Found 1021.1 (M+H).

Example 26 Preparation of Compound **32**

To compound **31** (125 mg, 0.123 mmol) in dry dichloromethane (10 ml) were added 1*H*-tetrazole (26 mg, 0.37 mmol) and dibenzyl diisopropylphosphoramidite (86.3 mg, 0.084 ml, 0.25 mmol). The mixture was stirred at room temperature for 30 min and then cooled to 0°C. m-Chloroperbenzoic acid (m-CPBA, 55%, 136 mg, 0.44 mmol) was added and the mixture was stirred for 30 min at 0°C. The mixture was then poured into 10% sodium hydrogen sulfite (20 ml) and extracted with dichloromethane (20 ml x 3). The organic layer was washed with saturated sodium bicarbonate solution (10 ml), dried with sodium sulfate and concentrated. The residue was purified by repeated flash chromatography (hexane : acetone, 6 : 1 and 4 : 1; toluene : acetone, 10 : 1 and 8 : 1) to give **32** (104 mg, 66%). TLC: R_f=0.20 (toluene : acetone, 8 : 1). $[\alpha]_D^{22}$=+23.7 (c 0.59, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (m, 9 H, 3 CH_3), 1.25 (m, 52 H, 26 CH_2), 1.44 (m, 2 H, CH_2), 1.63 (m, 2 H, CH_2), 1.74 (m, 2 H, CH_2), 2.14 (t, J=7.0 Hz, 2 H, CH_2), 2.22 (m, 2 H, CH_2), 2.31 (dd, J=15.0, 6.5 Hz, 1 H, Asp-β-H), 2.72 (dd, J=15.0, 3.5 Hz, 1 H, Asp-β'-H), 3.17 (m, 2 H, NCH_2), 3.68 (m, 2 H, 2 H-6), 3.95 (m, 1 H, H-5), 4.28

(ddd, J=10.5, 9.5, 3.5 Hz, 1 H, H-2), 4.47 (d, J=12.0, 1 H, CH*H*Ph), 4.55 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.60 (m, 3 H, H-4, Asp-α-H, CH*H*Ph), 4.72 (d, 1 H, J=11.5 Hz, 1 H, CH*H*Ph), 4.90 (m, 4 H, 2 CH_2Ph), 4.99 (d, J=3.5 Hz, 1 H, H-1), 5.34 (dd, J=10.5, 9.0 Hz, 1 H, H-3), 6.13 (d, J=9.5 Hz, 1 H, NH), 7.03 (t, J=5.5 Hz, 1 H, NH), 7.28 – 7.42 (m, 21 H, NH, Ar-H). Anal. calcd for $C_{75}H_{114}N_3O_{12}P$ (1280.72): C, 70.34; H, 8.97; N, 3.28; found: C, 70,54; H, 8.90; N, 3.26.

Example 27 Preparation of Compound **33**

To the solution of **32** (30 mg, 0.023 mmol) in a mixture of methanol : ethyl acetate : acetic acid (2 : 1 : 0.3, 80 ml), palladium on carbon (5%, 50 mg) was added. The mixture was stirred under hydrogen atmosphere at room temperature for 24 h. The solid was filtered off and the filtrate was concentrated. The residue was lyophilized from dioxane : chloroform (10 :1, 30 ml) to give **33** (17 mg, 79%). TLC: R_f=0.27 (chloroform : methanol : water, 2 : 1 : 0.1). $[\alpha]_D^{22}$=+310.8 (c0.074, chloroform : methanol, 4 : 1).

For the α-isomer: ^{1}H NMR (500 MHz, $CDCl_3$-CD_3OD, 1 : 1): δ 0.89 (t, J=7.0 Hz, 9 H, 3 CH_3), 1.28 (br. s, 52 H, 26 CH_2), 1.49 (m, 2 H, CH_2), 1.60 (m, 4 H, 2 CH_2), 2.23 (t, J=7.5 Hz, 2 H, CH_2), 2.35 (m, 2 H, CH_2), 2.49 (dd, J=14.0, 5.0 Hz, 1 H, Asp-β-H), 2.61 (dd, J=14.0, 6.0 Hz, 1 H, Asp-β'-H), 3.17 (m, 2 H, NCH_2), 3.72 (m, 2 H, 2 H-6), 3.96 (m, 1 H, H-5), 4.12 (dd, J=10.5, 3.5 Hz, 1 H, H-2), 4.28 (m, 1 H, H-4), 4.65 (m, 1 H, Asp-α-H), 5.10 (d, J=3.5 Hz, 1 H, H-1), 5.32 (dd, J=10.5, 9.5 Hz, 1 H, H-3). ^{31}P NMR (202.3 MHz, DMSO-d_6): δ –0.02 ppm. ES-MS calcd for $C_{47}H_{90}N_3O_{12}P$: 919.6. Found 921.1 (M+H).

Example 28 Preparation of Compound **35**

To a solution of **34**[31] (1.0 g, 3.50 mmol) in dry pyridine (10 ml), triphenylmethyl chloride (836 mg, 3.0 mmol) and DMAP (30.5 mg, 0.25 mmol) were added. The mixture was stirred at room temperature for 20 h. Additional trityl chloride (418 mg, 1.25 mmol) and DMAP (30.5 mg, 0.25 mmol) were added and the mixture was stirred at 40°C for 4 h. The solvent was removed by codistillation with toluene and the residue was purified by flash chromatography (hexane : ethyl acetate, 1 : 1) to give **35** (1.42 g, 88%). TLC: R_f=0.36 (hexane : ethyl acetate, 1 : 1). $[\alpha]_D^{22}$= -48.3 (c 0.6, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.70 (br. s, 1 H, OH), 3.00 (br s, 1 H, OH), 3.44 – 3.53 (m, 2 H, 2 H-6), 3.59 (m, 1 H, H-5), 3.65 (dd, J=9.5, 9.0 Hz, 1 H, H-4), 4.21 (dd, J=9.5, 8.0 Hz, 1 H, H-2), 4.55, 4.95 (2 d, J=12.0 Hz, each 1 H, CH_2Ph), 5.23 (d, J=8.0 Hz, 1

H, H-1), 7.10 – 7.80 (m, 24 H, Ar-H). Anal. calcd for $C_{40}H_{35}NO_7$•1.3 H_2O (641.72): C, 72.23; H, 5.70; N, 2.10. Found: C, 72.24; H, 5.92; N, 1.83.

Example 29 Preparation of Compound **36**

Sodium hydride (120 mg, 5.02 mmol) and benzyl bromide (0.86 g, 0.60 ml, 5.02 mmol) were added to dry DMF (10 ml). To this solution, a solution of **35** (1.34 g, 2.09 mmol) in DMF (8 ml) was added dropwise within 3 minutes. The mixture was stirred at room temperature for 1 h and treated further with an additional amount of benzyl bromide (0.43 g, 0.30 ml, 2.51 mmol) and sodium hydride (60 mg, 2.51 mmol). The reaction mixture was allowed to prolong for another 2 hours. Methanol (2 ml) was added and the mixture was stirred for 10 more minutes. The reaction was then poured into ice water (100 ml) and extracted with diethyl ether (60 ml x 3). Combined ether layers were washed with ice water (15 ml x 3), dried with sodium sulfate and concentrated. The residue was purified by flash chromatography (hexane : ethyl acetate, 5 : 1) to give **36** (1.55 g, 90%). TLC: R_f=0.60 (hexane : ethyl acetate, 3 : 1). $[\alpha]_D^{22}$= +5.5 (c 0.8, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.32 (dd, J=10.0, 4.5 Hz, 1 H, H-6a), 3.60 (m, 1 H, H-5), 3.68 (dd, J=10.0, 1.8 Hz, 1 H, H-6b), 4.03 (dd, J=9.5, 8.2 Hz, 1 H, H-2), 4.33 (m, 2 H, H-3, H-4), 4.43 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.47 (d, J=10.0 Hz, 1 H CH*H*Ph), 4.61 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.72 (d, J=10.0 Hz, 1 H, CH*H*Ph), 4.80 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.94 (d, J=12.0 Hz, 1 H, CH*H*Ph), 5.20 (d, J=8.2 Hz, 1 H, H-1), 6.84 – 7.80 (m, 34 H, Ar-H). Anal. calcd for $C_{54}H_{47}NO_7$•1.3 H_2O (821.97): C, 76.72; H, 5.91; N, 1.66. Found: C, 76.56; H, 6.13; N, 1.52.

Example 30 Preparation of Compound **37**

The solution of **36** (1.42 g, 1.73 mmol) in acetic acid – water (4 : 1, 60 ml) was stirred at 110°C for 1 h. The solvent was removed by codistillation with toluene and the residue was purified by flash chromatography (hexane : ethyl acetate, 2 : 1) to give **37** (700 mg, 70%). TLC: R_f=0.31 (hexane : ethyl acetate, 2 : 1). $[\alpha]_D^{22}$= +16.0 (c 0.25, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$) : δ 1.90 (dd, J=6.5, 6.5 Hz, 1 H, OH), 3.54 (m, 1 H, H-5), 3.73 (dd, J=9.5, 9.0 Hz, 1 H, H-4), 3.78 (m, 1 H, H-6a), 3.93 (m, 1 H, H-6b), 4.19 (dd, J=10.0, 8.5 Hz, 1 H, H-2), 4.36 (dd, J=10.0, 9.0 Hz, 1 H, H-3), 4.43 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.50 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.73 (d, J=11.0 Hz, 1 H, CH*H*Ph), 4.76 (J=12.0 Hz, 1 H, CH*H*Ph), 4.79 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.90 (d, J=11.0 Hz, 1 H, CH*H*Ph), 5.20 (d, J=8.5 Hz, 1 H, H-1), 6.80- 7.80 (m, 19 H, Ar-H).

Anal. calcd for $C_{35}H_{33}NO_7$•$0.8H_2O$ (579.65): C, 70.76; H, 5.87; N, 2.35. Found: C, 70.74; H, 6.14; N, 2.20.

Example 31 Preparation of Compound **38**

To the solution of **37** (0.60 g, 1.04 mmol) in 95% ethanol (40 ml) was added hydrazine monohydrate (2.06 g, 2.0 ml, 41.2 mmol). The mixture was refluxed for 2 h and then the solvent was removed in *vacuo*. The residue was purified by flash chromatography (1 to 2% methanol in dichloromethane) to give **38** (450 mg, 97%). TLC: R_f=0.20 (2% methanol in dichloromethane). $[\alpha]_D^{22}$= - 9.4 (c 0.35, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.75 (br. s, 3 H, OH, NH_2), 2.92 (dd, J=9.0, 8.0 Hz, 1 H, H-2), 3.43 (m, 1 H, H-5), 3.49 (dd, J=9.5, 9.5 Hz 1 H, H-4), 3.66 (dd, J=9.5, 9.0 Hz, 1 H, H-3), 3.76 (dd, J=12.0, 5.0 Hz, 1 H, H-6a), 3.91 (dd, J=12.0, 2.5 Hz, 1 H, H-6b), 4.39 (d, J=8.0 Hz, 1 H, H-1), 4.63 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.70 (d, J=11.0 Hz, 1 H, CH*H*Ph), 4.74 (d, J=11.0 Hz, 1 H, CH*H*Ph), 4.86 (d, J=11.0 Hz, 1 H, CH*H*Ph), 4.88 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.99 (d, J=11.0 Hz, 1 H, CH*H*Ph), 7.35 (m, 15 H, Ar-H). Anal. calcd for $C_{27}H_{31}NO_5$ (449.55): C, 72.14; H, 6.95; N, 3.15. Found: C, 72.34; H, 7.15; N, 3.12.

Example 32 Preparation of Compound **39**

Compound **38** (400 mg, 0.89 mmol), **15** (401 mg, 0.83 mmol) and DCC (275 mg, 1.34 mmol) were dissolved in dry dichloromethane (10 ml) and the resulting mixture was stirred at room temperature for 3 h. The solid was filtered off and washed with dichloromethane (4 ml). The filtrate was concentrated and the residue was purified by silica gel chromatography (0.5 to 1% methanol in dichloromethane) to give **39** (576 mg, 76%). TLC: R_f=0.30 (2% methanol in dichloromethane). $[\alpha]_D^{22}$= - 2.6 (c 0.7, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.25 (br. s, 42 H, 21 CH_2), 1.55 (m, 4 H, 2 CH_2), 2.14 (m, 2 H, CH_2), 2.28 (dd, J=15.0, 6.0 Hz, 1 H, CH*H*), 2.36 (dd, J=15.0, 6.0 Hz, 1 H, CH*H*), 3.48 (m, 2 H, H-2, H-5), 3.59 (dd, J=9.0, 9.0 Hz, 1 H, H-4), 3.71 (dd, J=11.5, 4.5 Hz, 1 H, H-6a), 3.87 (dd, J=11.5, 2.5 Hz, 1 H, H-6b),4.10 (dd, J=10.0, 9.0 Hz, 1 H, H-3), 4.60 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.65 (d, J=11.5 Hz, 2 H, 2 CH*H*Ph), 4.80 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.81 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.83 (d, J=12.0 Hz, 1H, CH*H*Ph), 4.95 (d, J=8.0 Hz, 1 H, H-1), 5.04 (m, 1 H, lipid-3-H), 5.90 (d, J=8.0 Hz, 1 H, NH), 7.35 (m, 15 H, Ar-H). Anal. calcd for $C_{57}H_{87}NO_8$•$0.3H_2O$ (914.32): C, 74.44; H, 9.60; N, 1.52. Found: C, 74.38; H, 9.85; N, 1.56.

Example 33 Preparation of compound **40**

To the solution of compound **38** (410 mg, 0.913 mmol) in dry dichloromethane (30 ml), compound **14** (623 mg, 1.37 mmol) and DCC (564 mg, 2.74 mmol) were added. The mixture was stirred at room temperature for 24 hours. The solid was filtered off and washed with dichloromethane (4 ml). The filtrate was concentrated and the residue purified by silica gel chromatography (0.5 to 1% methanol in dichloromethane) to give **40** (664 mg, 82%). TLC: R_f=0.33 (2% 1% methanol in dichloromethane). $[\alpha]_D^{22}$= - 3.2 (c 0.6, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, J=7.0 Hz, 6 H, 2 CH_3), 1.25 (m, 38 H, 19 CH_2), 1.55 (m, 4 H, 2 CH_2), 1.89 (dd, J=7.0, 6.0 Hz, 1 H, OH), 2.15 (m, 2 H, CH_2), 2.27 (dd, J=15.0, 5.5 Hz, 1 H, CH*H*), 2.36 (dd, J=15.0, 6.0 Hz, 1 H, CH*H*), 3.46 (m, 1 H, H-5), 3.52 (m, 1 H, H-4), 3.59 (dd, J=10.0, 9.0 Hz, 1 H, H-3), 3.70 (m, 1 H, H-6a), 3.86 (m, 1 H, H-6b), 4.10 (dd, J=10.0, 8.0 Hz, 1 H, H-2), 4.60 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.64 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.65 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.81 (d, J=11.5 Hz, 2 H, 2 CH*H*Ph), 4.83 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.95 (d, J=8.0 Hz, 1 H, H-1), 5.04 (m, 1 H, lipid-3-H), 5.92 (d, J=8.0 Hz, 1 H, NH), 7.30 (m, 15 H, Ar-H). Anal. calcd for : $C_{55}H_{83}NO_8$ (886.26): C, 74.47; H, 9.44; N, 1.58. Found: C, 74.25; H, 9.44; N, 1.64.

Example 34 Preparation of Compound **41**

Compound **28** (312 mg, 0.65 mmol), **14** (200 mg, 0.44 mmol), DCC (136 mg, 0.66 mmol) and DMAP (27 mg, 0.22 mmol) were dissolved in dry dichloromethane (5 ml). The mixture was stirred at room temperature for 4 h. The solid was filtered off and washed with ethyl acetate (5 ml). The filtrate was concentrated and the residue was purified by flash chromatography (hexane : ethyl acetate, 8 : 1) to give **41** (398 mg, 98%). TLC: R_f=0.69 (hexane : ethyl acetate, 3 : 1). $[\alpha]_D^{22}$= +32.0 (c 0.5, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.25 (m, 38 H, 19 CH_2), 1.52 (m, 4 H, 2 CH_2), 2.16 (t, J=7.5 Hz, 2 H, CH_2), 2.50 (dd, J=16.0, 6.0 Hz, 1 H, CH*H*), 2.63 (dd, J=16.0, 6.0 Hz, 1 H, CH*H*), 3.71 (dd, J=9.5, 9.5 Hz, 1 H, H-4), 3.78 (dd, J=10.0, 10.0 Hz, 1 H, H-6a), 3.94 (m, 1 H, H-5), 3.98 – 4.08 (m, 2 H, H-2, CH*H*CH=CH_2), 4.21 (m, 1 H, CH*H*CH=CH_2), 4.29 (dd, J=10.0, 5.0 Hz, 1 H, H-6b), 4.69, 4.76 (2 d, J=12.0 Hz, each 1 H, Troc-CH_2), 4.94 (d, J=3.6 Hz, 1 H, H-1), 5.16 (m, 1 H, lipid-3-H), 5.30 (m, 2 H, CH=C*H_2*), 5.39 (dd, J=9.5, 9.5 Hz, 1 H, H-3), 5.42 (d, J=10.0 Hz, 1 H, NH), 5.53 (s, 1

H, C*H*Ph), 5.90 (m, 1 H, C*H*=CH_2), 7.30 – 7.35 (m, 15 H, Ar-H). Anal. calcd for $C_{47}H_{74}Cl_3NO_{10}$ (919.46): C, 61.40; H, 8.11; N, 1.52. Found: C, 61.40; H, 8.19; N, 1.58.

Example 35 Preparation of Compound **42**

[Bis(methyldiphenylphosphine)](1,5-cyclooctadiene) iridium(I) hexafluorophosphate (37 mg, 0.044 mmol) was suspended in dry THF (5 ml) and hydrogen gas was bubbled in for 5 min to give a yellowish solution, which was added to the solution of **41** (400 mg, 0.44 mmol) in dry THF (5 ml). The mixture was stirred at room temperature for 2 hours. Water (0.5 ml) and N-bromosuccinimide (NBS, 117 mg, 0.66 mmol) were then added and the reaction was stirred for 1 hour longer. Remainder obtained from solvent removal was dissolved in ethyl acetate (200 ml) and washed with saturated sodium bicarbonate solution (20 ml x 2). Combined organic layers were dried with sodium sulfate and concentrated. The residue was purified by flash chromatography (hexane : ethyl acetate, 4 : 1 and 3 : 1) to give **42** (314 mg, 82%) as an anomeric mixture in a ratio of $\alpha/\beta = 4 : 1$. TLC: R_f=0.36 (hexane : ethyl acetate, 3 : 1) $[\alpha]_D^{22}$= - 9.6 (c 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$) for the α-isomer: δ 0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.24 (m, 38 H, 19 CH_2), 1.50 (m, 4 H, 2 CH_2), 2.16 (t, J=7.5 Hz, 2 H, CH_2), 2.49 (dd, J=15.0, 5.0 Hz, 1 H, CH*H*), 2.60 (dd, J=15.0, 7.0 Hz, 1 H, CH*H*), 3.65 (d, J=4.0 Hz, 1 H, OH), 3.70 (dd, J=9.5, 9.5 Hz, 1 H, H-4), 3.77 (dd, J=10.0, 10.0 Hz, 1 H, H-6a), 4.03 (m, 1 H, H-2), 4.17 (m, 1 H, H-5), 4.28 (dd, J=10.0, 4.5 Hz, 1 H, H-6b), 4.67, 4.75 (2 d, J=12.0 Hz, each 1 H, Troc-CH_2), 5.15 (m, 1 H, lipid-3-H), 5.35 (dd, J=4.0, 4.0 Hz, 1 H, H-1), 5.43 (dd, J=9.5, 9.5 Hz, 1 H, H-3), 5.51 (s, 1 H, C*H*Ph), 5.81 (d, J=10.0 Hz, 1 H, NH), 7.32 – 7.47 (m, 5 H, Ar-H). Anal. calcd for $C_{44}H_{70}Cl_3NO_{10}$ (879.39): C, 60.10; H, 8.02; N, 1.59. Found: C, 60.11; H, 8.09; N, 1.61.

Example 36 Preparation of Compound **43**

To a solution of **42** (2.50 g, 2.88 mmol) in dry dichloromethane (30 ml), trichloroacetonitrile (8.64 g, 6.0 ml, 60.0 mmol) and DBU (10 drops) were added. The mixture was stirred at room temperature for 2 h and concentrated in *vacuo* (not to dryness). The residue was purified by flash chromatography (hexane : ethyl acetate : triethylamine, 6 : 1 : 1% and 5 : 1 : 15) to give **43** (2.40 g, 81%). TLC: R_f=0.25 (hexane : ethyl acetate, 8 : 1). $[\alpha]_D^{22}$= +35.0 (c 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, J=7.0 Hz, 6 H, 2 CH_3), 1.25 (m, 38 Hz, 19 CH_2), 1.50 (m, 4 H, 2 CH_2), 2.20 (t, J=7.5 Hz, 2 H, CH_2), 2.56 (dd, J=15.5, 5.5 Hz, 1 H, CH*H*), 2.65 (dd, J=15.5,

7.0 Hz, 1 H, CH*H*), 3.81 (dd, J=10.0, 10.0 Hz, 1 H, H-4), 3.83 (dd, J=10.0, 10.0 Hz, 1 H, H-6a), 4.06 (m, 1 H, H-5), 4.25 (ddd, J=10.0, 9.0, 4.0 Hz, 1 H, H-2), 4.36 (dd, H=10.0, 5.0 Hz, 1 H, H-6b), 4.63, 4.78 (2 d, J=12.0 Hz, each 1 H, Troc-CH_2), 5.18 (m, 1 H, lipid-3-H), 5.45 (dd, J=10.0, 10.0 Hz, 1 H, H-3), 5.56 (d, J=9.0 Hz, 1 H, NH), 5.58 (s, 1 H, C*H*Ph), 6.42 (d, J=4.0 Hz, 1 H, H-1), 7.30 – 7.45 (m, 5 H, Ar-H), 8.73 (s, H, NH). Anal. calcd for $C_{46}H_{70}Cl_6N_2O_{10}$ (1023.78): C, 53.97; H, 6.89; N, 2.74. Found: C, 53. 80; H, 6.77; N, 2.80.

Example 37 Preparation of Compound **44**

To the solution of **39** (269 mg, 0.295 mmol) and **43** (452 mg, 0.442 mmol) in dry dichloromethane (6 ml) was added molecular sieves (4 A, 1.0 g). The mixture was stirred under nitrogen at room temperature for 20 min and then cooled to 0°C. Trifluoroboron etherate solution (0.15 M in CH_2Cl_2, 0.5 ml) was added dropwise and reaction mixture was stirred at 0°C for 30 minutes. It was then treated with triethylamine (0.05 ml). The solid was filtered off and washed with dichloromethane. The filtrate was concentrated and the residue was purified by precipitation from ethyl acetate and by silica gel chromatography (1.5% methanol in dichloromethane) to give **44** (340 mg, 65%). TLC: R_f=0.31 (2% methanol in dichloromethane). $[\alpha]_D^{22}$= - 13.3 (c 0.7, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD): δ 0.88 (t, J=6.5 Hz, 12 H, 4 CH_3), 1.24 (m, 72 H, 36 CH_2), 1.52 (m, 8 H, 4 CH_2), 1.70 (m, 4 H, 2 CH_2), 1.94 (m, 4 H, 2 CH_2), 2.16 (m, 4 H, 2 CH_2), 2.27 (dd, J=15.0, 6.0 Hz, 1 H, CH*H*), 2.35 (dd, J=15.0, 6.5 Hz, 1 H, CH*H*), 2.49 (dd, J=15.5, J=5.5 Hz, 2 H, CH*H*), 2.59. (dd, J=15.5, 7.0 Hz, 1 H, CH*H*), 3.35 – 4.14 (m, 10 H, H-2, H-3, H-4, H-5, 2 H-6, H-2', H-4', H-5', H-6'a), 4.31 (dd, J=10.5, 5.5 Hz, 1 H H-6'b), 4.53 (d, J=8.0 Hz, 1 H, H-1'), 4.57 – 4.69 (m, 5 H, Cl_3CCH_2, 3 CH*H*Ph), 4.75 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.78 (d, J=11.0, 1 H, CH*H*Ph), 4.88 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.89 (d, J=8.0 Hz, 1 H, H-1), 5.04 (m, 1 H, lipid-3-H), 5.18 (m, 2 H, H-3', lipid-3-H), 5.48 (s, 1 H, C*H*Ph), 7.25 – 7.45 (m, 20 H, Ar-H). Anal. calcd for $C_{101}H_{155}Cl_3N_2O_{17}•2H_2O$ (1775.70): C, 66.95; H, 8.84; N, 1.55. Found: C, 66.83; H, 8.63; N, 1.66.

Example 38 Preparation of Compound **45**

Using the same procedure described for the preparation of **30**, compound **45** was synthesized. Initially crude amine was obtained from **44** (224 mg, 0.126 mmol) treated with activated Zinc (5.0 g) and 80% acetic acid in ethyl acetate (500 ml) for 60 hours at room temperature. It was

lyophilized from dioxane to give the amino compound (192 mg, 95%). Later, this amine compound (212 mg, 0.132 mmol) and **13** (115 mg, 0.238 mmol) in the presence of DCC (122 mg, 0.60 mmol) and dry dichloromethane (10 mg) were converted to compound **45** in 48 hours at room temperature. Pure **45** (160 mg, 60%) was obtained from silica gel chromatographic purification (3% acetone in chloroform) of crude **45**. TLC: R_f=0.30 (2% methanol in dichloromethane). $[\alpha]_D^{22}$= - 8.9 (c 0.73, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J=6.5 Hz, 18 H, 6 CH_3), 1.10 – 1.90 (m, 126 H, 63 CH_2), 2.14 (t, J=7.0 Hz, 4 H, 2 CH_2), 2.26 (m, 6 H, 3 CH_2), 2.50 (dd, J=15.5, 5.5 Hz, 1 H, CH*H*), 2.59 (dd, J=15.5, 7.5 Hz, 1 H, CH*H*), 3.40 – 4.14 (m, 10 H, H-2, H-3, H-4, H-5, 2 H-6, H-2', H-4', H-5', H-6'a), 4.27 (dd, J=11.0, 5.0 Hz, 1 H, H-6'b), 4.60 (d, J=11.5 Hz, 2 H, 2 CH*H*Ph), 4.66 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.73 (d, J=8.0 Hz, 1 H, H-1'), 4.74 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.75 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.82 (d, J=8.0 Hz, 1 H, H-1), 4.86 (d, J=12.0 Hz, 1 H, CH*H*Ph), 5.05 (m, 2 H, 2 lipid-3-H), 5.15 (m, 1 H, lipid-3-H), 5.25 (dd, J=10.0, 10.0 Hz, 1 H, H-3'), 5.48 (s, 1 H, C*H*Ph), 5.94 (d, J=9.0 Hz, 1 H, NH), 6.06 (d, J=9.0 Hz, 1 H, NH), 7.25 – 7.43 (m, 20 H, Ar-H). Anal. calcd for $C_{124}H_{202}N_2O_{18}\bullet1.5H_2O$ (2008.96): C, 73.15; H, 10.14; N, 1.37. Found: C, 73.06; H, 9.95; N, 1.22.

Example 39 Preparation of Compound **46**

To a solution of **45** (148 mg, 0.074 mmol) in dry THF (8 ml) was added molecular sieves (4 A, 1.0 g). The mixture was stirred at room temperature under nitrogen for 20 min. Sodium cyanoborohydride (340 mg, 5.41 mmol) was added and the mixture was cooled to 0°C. HCl (g) / Et_2O solution (~ 3 ml) was added dropwise slowly till no gas was evolved. The mixture was then poured into saturated sodium bicarbonate solution (50 ml) and extracted with dichloromethane (100 ml x 3). Combined organic layers were washed with saturated sodium chloride solution (20 ml) and dried with sodium sulfate, and concentrated. The residue was purified by flash chromatography (gradient elution with chloroform : acetone , from 100 : 2 to 100 : 5) to give **46** (79 mg, 53%). TLC: R_f=0.16 (chloroform : acetone, 100 : 5). $[\alpha]_D^{22}$= - 16.0 (c 0.2, chloroform). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (t, J=6.5 Hz, 18 H, 6 CH_3), 1.08 – 1.94 (m, 126 H, 63 CH_2), 2.13 – 2.36 (m, 10 H, 5 CH_2), 2.51 (dd, J=15.0, 5.0 Hz, 1 H, CH*H*), 2.58 (dd, J=15.0, 8.0 Hz, 1 H, CH*H*), 3.32 (br. s, 1 H, OH), 3.43 – 3.77 (m, 8 H, H-2, H-4. H-5, 2 H-6, H-4', H-5', H-6'a), 3.86 (ddd, J=10.0, 8.5, 8.5 Hz, 1 H, H-2'), 3.92 (dd, J=9.0, 9.0 Hz, 1 H, H-3),

4.08 (dd, J=11.0, 2.5 Hz, 1 H, H-6'b), 4.61 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.55 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.56 (d, J=8.5 Hz, 1 H, H-1'), 4.57 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.60 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.64 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.72 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.73 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.79 (d, J=7.5 Hz, 1 H, H-1), 4.83 (d, J=12.5 Hz, 1 H, CH*H*Ph), 4.95 (dd, J=10.5, 9.0 Hz, 1 H, H-3'), 4.99 – 5.08 (m, 2 H, 2 lipid-3-H), 5.11 (m, 1 H, lipid-3-H), 5.77 (d, J=8.5 Hz, 1H, NH), 5.93 (d, J=8.5 Hz, 1 H, NH), 7.30 (m, 20 H, Ar-H). Anal. calcd for $C_{124}H_{204}N_2O_{18}$ (2010.98): C, 74.06; H, 10.22; N, 1.39. Found: C, 73.74; H, 10.57; N, 1.43.

Example 40 Preparation of Compound **47**

By following the same procedure described for the preparation of **32**, compound **47** was obtained from the reaction of **46** (68 mg, 0.034 mmol) with 1*H*-tetrazole (10 mg, 0.144 mmol), dibenzyl diisopropylphosphoramidite (33 mg, 0.032 ml, 0.096 mmol) and m-chloroperbenzoic acid (59 mg, ~55%, 0.19 mmol) in dry dichloromethane (3 ml). After stirring for 1 hour at 0°C, the work up rendered residue which was purified by repeated silica gel chromatography (gradient elution with 1 to 5% acetone in chloroform and toluene : acetone, from 18 : 1 to 12 : 1) gave **47** (65 mg, 85%). TLC: R_f=0.22 (5% acetone in chloroform). $[\alpha]_D^{22}$= - 4.0 (c 0.4, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (m, 18 H, 6 CH3), 1.25 (m, 114 H, 57 CH_2), 1.45 – 1.70 (m, 12 H, 6 CH_2), 2.10 – 2.50 (m, 12 H, 6 CH_2), 3.52 – 3.93 (m, 9 H, H-2, H-3, H-4, H-5, H-6a, H-2', H-5', 2 H-6'), 4.09 (br. d, J=11.0 Hz, 1 H, H-6b), 4.43 (m, 3 H, CH_2Ph, H-4'), 4.56 – 4.91 (m, 12 H, 6 CH_2Ph), 4.78 (d, J=7.5 Hz, 1 H. H-1'), 4.98 (d, J=8.5 Hz, 1 H, H-1), 5.05 (m, 2 H, 2 lipid-3-H), 5.16 (m, 1 H, lipid-3-H), 5.39 (dd, J=10.0, 9.0 Hz, 1 H, H-3'), 5.88 (d, J=8.5 Hz, 1 H, NH), 6.08 (d, J=7.5 Hz, 1 H, NH), 7.25 (m, 30 H, Ar-H). Anal. calcd for $C_{138}H_{217}N_2O_{21}P$ (2271.21): C, 72.98; H, 9.63; N, 1.23. Found: C, 72.83; H, 9.60; N, 1.23.

Example 41 Preparation of Compound **48**

To a solution of **47** (48 mg, 0.021 mmol) in THF – HOAc (10 : 1, 90 ml) was added palladium on carbon (5%, 70 mg). The mixture was stirred at room temperature under hydrogen atmosphere for 24 h. The solid was filtered off and the filtrate was concentrated in *vacuo*. The residue was purified by flash chromatography (chloroform : methanol : water, 4 : 1 : 0 and then 3 : 1 : 0.1) to give **48** (25 mg, 68%). TLC: R_f=0.38 (chloroform : methanol : water, 3 : 1 : 0.1).

$[\alpha]_D^{22}$= +8.0 (c 0.1, chloroform : methanol, 4 : 1). ES-MS calcd for $C_{96}H_{181}N_2O_{21}P$: 1729.3. Found: 1728.3 (M-H) (negative mode).

Example 42 Preparation of Compound **49**

To a solution of **40** (290 mg, 0.328 mmol) and **43** (503 mg, 0.492 mmol) in dry dichloromethane (6 ml) was added molecular sieves (4 A, 0.5 g). The mixture was stirred under nitrogen at room temperature for 20 min. Trifluoroboron etherate solution (0.1 M in CH_2Cl_2, 1.3 ml) was added dropwise within 20 min. The mixture was stirred for 1 h and then poured into saturated sodium bicarbonate solution (10 ml) and extracted with dichloromethane (20 ml x 3). Combined organic layers were dried with sodium sulfate and concentrated. The residue was purified by silica gel chromatography (0.5 to 1% methanol in dichloromethane) to give 49 (457 mg, 80%). TLC: R_f=0.21 (3% acetone in chloroform). $[\alpha]_D^{22}$= - 17.8 (c 0.6, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, J=7.0 Hz, 12 H, 4 CH_3), 1.25 (m, 76 H, 38 CH_2), 1.52 (m, 8 H, 4 CH_2), 2.15 (m, 4 H, 2 CH_2), 2.26, 2.35 (2 dd, J=14.0, 6.0 Hz, each 1 H, CH_2), 2.48 (dd, J=15.0, 5.5 Hz, 1 H, CH*H*), 2.58 (dd, J=15.0, 7.0 Hz, 1 H, CH*H*), 3.34 – 3.78 (m, 8 H, H-2, H-3, H-4, H-5, H-6a, H-2', H-4', H-6'a), 4.02 – 4.13 (m, 2 H, H-6b, H-5'), 4.30 (dd, J=10.5, 5.0 Hz, 1 H, H-6'b), 4.52 (d, J=8.0 Hz, 1 H, H-1'), 4.57 – 4.90 (m, 8 H, 3 C*H*$_2$Ph, Troc-CH_2), 4.89 (d, J=8.0 Hz, 1 H, H-1) 5.02 (m, 1 H, lipid-3-H), 5.15 (m, 3 H, NH, H-3', lipid-3-H), 5.55 (s, 1 H, C*H*Ph), 6.00 (d, J=8.0 Hz, 1 H, NH), 7.25 - 7.45 (m, 20 H, Ar-H). Anal. calcd for $C_{99}H_{151}Cl_3N_2O_{17}$ (1747.64): C, 68.04; H, 8.71; N, 1.60. Found: C, 67.92, H, 8.85, N, 1.64.

Example 43 Preparation of Compound **50**

Compound **50** was synthesized from **49** (740 mg, 0.424 mmol) treated with activated Zinc (5.0 g, 76.5 mmol) and 80% acetic acid in ethyl acetate (400 ml) at room temperature for 60 hours as its described for the preparation of **13**. Crude **50** (666 mg, 100%) obtained from solvent removal was lyophilized from dioxane and used without any further purification.

Example 44 Preparation of Compound **51**

In a similar method as described for **45**, compound **50** (175 mg, 0.11 mmol) was coupled with **14** (101 mg, 0.22 mmol) in presence of DCC (68 mg, 0.33 mmol) in dry dichloromethane (5 ml). After usual work-up and silica gel chromatography (1% methanol in dichloromethane) gave **51**

(150 mg, 67%). TLC: R_f=0.27 (2% methanol in dichloromethane). $[\alpha]_D^{22}$= - 14.2 (c 0.5, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, J=7.0 Hz, 18 H, 6 CH_3), 1.25 (m, 114 H, 57 CH_2), 1.53 (m, 12 H, 6 CH_2), 2.15 (t, J=7.0 Hz, 4 H, 2 CH_2), 2.23 – 2.39 (m, 6 H, 3 CH_2), 2.56 (dd, J=15.5, 5.5 Hz, 1 H, CH*H*), 2.60 (dd, J=15.5, 7.0 Hz, 1 H, CH*H*), 3.37 – 4.00 (m, 9 H, H-2, H-3, H-4, H-5, H-6a, H-2', H-4', H-5', H-6'a), 4.09 (dd, J=11.0, 2.0 Hz, 1 H, H-6b), 4.27 (dd, J=11.0, 4.5 Hz, 1 H, H-6'b), 4.58 – 4.88 (m, 7 H, 3 C*H*$_2$Ph, H-1'), 4.82 (d, J=7.5 Hz, 1 H, H-1), 5.00 – 5.09 (m, 2 H, 2 lipid-3-H), 5.16 (m, 1 H, lipid-3-H), 5.26 (dd, J=10.0, 10.0 Hz, 1 H, H-3'), 5.47 (s, 1 H, C*H*Ph), 5.93 (d, J=8.5 Hz, 1 H, NH), 6.06 (d, J=8.0 Hz, 1 H, NH), 7.25 – 7.45 (m, 20 H, Ar-H). Anal. calcd for $C_{124}H_{202}N_2O_{18}\bullet 0.5H_2O$ (2008.96): C, 73.80; H, 10.14; N, 1.39. Found: C, 73.64; H, 9.88; N, 1.41.

Example 45 Preparation of Compound **52**

In a similar method as described for **31**, compound **51** (135 mg, 0.067 mmol) was treated with sodium cyanoborohydride (211 mg, 3.36 mmol) and HCl (g) / Et_2O in dry THF : $CHCl_3$ (4 : 1, 50 ml) at room temperature. After usual work-up and flash chromatography (2 to 5% acetone in chloroform) afforded **52** (112 mg, 83%). TLC: R_f=0.20 (2% methanol in dichloromethane). $[\alpha]_D^{22}$= - 13.5 (c 0.6, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J=6.5 Hz, 18 H, 6 CH_3), 1.25 (m, 114 H, 57 CH_2), 1.50 (m, 12 H, 6 CH_2), 2.14 (t, J=7.0 Hz, 2 H, CH_2), 2.23 – 2.60 (m, 10 H, 5 CH_2), 3.33 (d, J=3.3 Hz, 1 H, OH), 3.44 – 3.96 (m, 10 H, H-2, H-3, H-4, H-5, H-6a, H-2', H-4', H-5', 2 H-6'), 4.09 (dd, J=10.0, 2.0 Hz, 1 H, H-6b), 4.49 – 4.86 (m, 9 H, 4 C*H*$_2$Ph, H-1'), 4.80 (d, J=7.5 Hz, 1 H, H-1), 4.92 – 5.18 (m, 4 H, H-3', 3 lipid-3-H), 5.80 (d, J=9.0 Hz, 1 H NH), 5.95 (d, J=8.5 Hz, 1 H, NH), 7.30 (m, 20 H, Ar- H). Anal. calcd for $C_{124}H_{204}N_2O_{18}\bullet H_2O$ (2010.98): C, 73.40; H, 10.23; N, 1.38. Found: C, 73.40; H, 10.04; N, 1.38.

Example 46 Preparation of Compound **53**

In a similar method as described for **32**, compound **52** (61 mg, 0.030 mmol) was treated with 1*H* tetrazole (12.6 mg, 0.18 mmol) and dibenzyl diisopropylphosphoramidite (42 mg, 0.041 ml, 0.12 mmol) in dry dichloromethane (3.0 ml) and then subsequently with m-CPBA (75 mg, 55%, 0.24 mmol). After usual work-up and silica gel chromatography (1 to 5% acetone in chloroform and then toluene: acetone, from 18:1 to 12:1) afforded **53** (58 mg, 85%). TLC: R_f=0.17 (1% acetone in chloroform). $[\alpha]_D^{22}$= - 3.1 (c 0.35, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, J=6.5

Hz, 18 H, 6 CH_3), 1.24 (m, 114 H, 57 CH_2), 1.40 – 1.57 (m, 12 H, 6 CH_2), 2.11 – 2.50 (m, 12 H, 6 CH_2), 3.52 – 3.94 (m, 9 H, H-2, H-3, H-4, H-5, H-6a, H-2', H-5', 2 H-6'), 4.09 (dd, J=11.0, 2.0 Hz, 1 H, H-6b), 4.44 (m, 3 H, C*H*$_2$Ph, H-4'), 4.56 – 4.90 (m, 12 H, 6 C*H*$_2$Ph), 4.78 (d, J=8.0 Hz, 1 H, H-1'), 4.98 (d, J=8.0 Hz, 1 H, H-1), 5.05 (m, 2 H, 2 lipid-3-H), 5.16 (m, 1 H, lipid-3-H), 5.39 (dd, J=10.0, 9.0 Hz, 1 H, H-3'), 5.88 (d, J=8.5 Hz, 1 H, NH), 6.08 (d, J=8.0 Hz, 1 H, NH), 7.25 (m, 30 H, Ar-H). Anal. calcd for $C_{138}H_{217}N_2O_{21}P{\bullet}0.5H_2O$ (2271.21): C, 72.69; H, 9.63; N, 1.22. Found: C, 72.45; H, 9.32; N, 1.19.

Example 47 Preparation of Compound **54**

In a similar method as described for **33**, compound **53** (54 mg, 0.028 mmol) was converted to **54** (30 mg, 62%) using palladium on carbon (5%, 70 mg) in THF : acetic acid, 10:1 (90 ml) in hydrogen atmosphere. TLC: R_f=0.35 (chloroform : methanol : water, 3 : 1 : 0.1). $[\alpha]_D^{22}$= - 10.0 (c 0.1, chloroform : methanol, 4 : 1). ES-MS calcd for $C_{96}H_{181}N_2O_{21}P$: 1729.3. Found: 1728 (M-H) (negative mode).

Example 48 Preparation of Compound **55**

In a similar method as described for **30**, compound **50** (300 mg, 0.19 mmol) was coupled with **5** (222 mg, 0.475 mmol) in dichloromethane : DMF (4:1, 50 ml). Silica gel chromatography (2 to 6% acetone in chloroform) gave **55** (256 mg, 66%). TLC: R_f=0.33 (chloroform : acetone, 9 : 1). $[\alpha]_D^{22}$= - 24.0 (c 0.2, chloroform : methanol, 5 : 1). ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD, 4 : 1): δ 0.98 (t, J=6.5 Hz, 18 H, 6 CH_3), 1.25 (m, 108 H, 54 CH_2), 1.40 – 1.60 (m, 14 H, 7 CH_2), 2.07 – 2.63 (m, 12 H, 6 CH_2), 3.12 (t, J=7.0 Hz, 2 H, NCH_2), 3.45 – 3.90 (m, 9 H, H-2, H-3, H-4, H-5, H-6a, H-2', H-4', H-5', H-6'a), 4.10 (dd, J=11.0, 2.0 Hz, 1 H, H-6b), 4.31 (dd, J=11.0, 5.5 Hz, H-6'b), 4.48 (t, J=6.5 Hz, 1 H, Asp-α-H), 4.56 – 4.87 (m, 7 H, 3 C*H*$_2$Ph, H-1'), 4.85 (d, J=8.0 Hz, 1 H, H-1), 5.08 – 5.19 (m, 2 H, 2 lipid-3-H), 5.34 (dd, J=10.0, 10.0 Hz, 1 H, H-3'), 5.51 (s, 1 H, C*H*Ph), 7.20 – 7.45 (m, 20 H, Ar-H). Anal. calcd for $C_{123}H_{200}N_4O_{18}{\bullet}H_2O$ (2022.95): C, 72.38; H, 9.97; N, 2.74. Found: C, 72.19; H, 9.68; N, 2.70.

Example 49 Preparation of Compound **56**

In a similar method as described for **31**, compound **55** (220 mg, 0.109 mmol) was treated with sodium cyanoborohydride (1.37 g, 21.78 mmol) and HCl (g) / Et_2O in dry THF – $CHCl_3$ (4 : 1,

50 ml) at room temperature to afford **56** (150 mg, 68%). TLC: R_f=0.20 (2 % methanol in dichloromethane). $[\alpha]_D^{22}$= - 14.0 (c 0.2, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$ - CD_3OD, 4 : 1): δ 0.72 (t, J=6.5 Hz, 18 H, 6 CH_3), 1.10 (m, 108 H, 54 CH_2), 1.21 – 1.46 (m, 14 H, 7 CH_2), 1.96 - 2.39 (m, 12 H, 6 CH_2), 2.15 (d, J=6.0 Hz, 1 H, OH), 2.95 (m, 2 H, NCH_2), 3.30 – 3.77 (m, 10 H, H-2, H-3, H-4, H-5, H-6a, H-2', H-4', H-5', 2 H-6'), 3.96 (dd, J=11.0, 2.0 Hz, 1 H, H-6b), 4.31 (m, 1 H, Asp-α-H), 4.35 – 4.68 (m, 9 H, 4 C*H*$_2$Ph, H-1), 4.48 (d, J=8.0 Hz, 1 H, H-1'), 4.86 (dd, J=10.0, 9.0 Hz, 1 H, H-3'), 4.96 (m, 2 H, 2 lipid-3-H), 7.10 (m, 20 H, Ar-H). Anal. calcd for $C_{123}H_{202}N_4O_{18}$•1.5H_2O (2024.96): C, 72.00; H, 10.07; N, 2.73. Found: C, 71.97; H, 9.91; N, 2.69.

Example 50 Preparation of Compound **57**

In a similar method as described for **32**, compound **56** (85 mg, 0.042 mmol) was converted to **57** (80 mg, 83%). TLC: R_f=0.37 (chloroform : acetone, 9 : 1). $[\alpha]_D^{22}$= - 6.2 (c 0.4, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (m, 18 H, 6 CH_3), 1.23 (m, 108 H, 54 CH_2), 1.25 – 1.60 (m, 14 H, 7 CH_2), 2.12 – 2.57 (m, 12 H, 6 CH_2), 3.15 (m, 2 H, NCH_2), 3.48 – 3.92 (m, 9 H, H-2, H-3, H-4, H-5, H-6a, H-2', H-5', 2 H-6'), 4.10 (br. d, J=11.0 Hz, 1 H, H-6b), 4.37 – 4.90 (m, 16 H, H-1, H-1', H-4', Asp-α-H, 6 C*H*$_2$Ph), 5.08 (m, 2 H, 2 lipid-3-H), 5.32 (dd, J=10.0, 9.0 Hz, 1 H, H-3'), 6.20 (d, J=8.0 Hz, 1 H, NH), 6.62 (d, J=8.0 Hz, 1 H, NH), 7.08 (m, 2 H, 2 NH), 7.25 (m, 30 H, Ar-H). Anal. calcd for $C_{137}H_{215}N_4O_{21}P$•1.5H_2O (2285.19): C, 71.16; H, 9.50; N, 2.42. Found: C, 71.02; H, 9.43; N, 2.23.

Example 51 Preparation of Compound **58**

In a similar method as described for **33**, compound **57** (52 mg, 0.023 mmol) was converted to **58** (32 mg, 80%). TLC: R_f=0.29 (chloroform : methanol : water, 3 : 1 : 0.1). $[\alpha]_D^{22}$= -10.0 (c 0.1, chloroform : methanol, 4 : 1). ES-MS calcd for $C_{95}H_{179}N_4O_{21}P$: 1743.3. Found: 1742 (M-H) (negative mode).

Example 52 Preparation of Compound **60**

(1) Compound **59**: To a solution of **34** (15.95 g, 40.13 mmol) in dry acetonitrile (250 ml), benzaldehyde dimethyl acetal (18.32 g, 18.01 ml, 120.4 mmol) and p-toluenesulfonic acid (328 mg, 1.73 mmol) were added. After stirring at room temperature and under nitrogen atmosphere

for 1.5 hours, reaction was terminated by an addition of triethyl amine (2 ml). It was then concentrated in *vacuo* and the residue was purified by silica gel chromatography (hexane : ethyl acetate, 7 : 3) to give **59** (13.93 g, 71%).

(2) Solution of **59** (2.02 g, 4.14 mmol) in dry DMF (15 ml) was added dropwise within 10 min to a mixture of sodium hydride (230 mg, 9.58 mmol), allyl bromide (0.75 g, 0.50 ml, 6.21 mmol) and dry DMF (20 ml). The reaction mixture was then stirred at room temperature for 3 hours. Similar work-up as described for **36** and purification by silica gel chromatography (hexane : ethyl acetate, 5 : 1) gave the 3-O-allyl compound (1.79 g, 82%).

(3) The 3-O-allyl compound (5.79 g, 11.0 mmol) was treated with acetic acid – water (4 : 1, 130 ml) at 65°C for 6 h. The solvent was removed and the residue was purified by flash chromatography (hexane : ethyl acetate, 1 : 2) to give 4,6-dihydroxy compound (4.91 g, 95%).

(4) Compound **60**: To a solution of the above 4,6-dihydroxy compound (4.79 g, 10.91 mmol) in dry pyridine (50 ml), triphenylmethyl chloride (6.38 g, 22.86 mmol) and DMAP (266 mg, 2.18 mmol) were added. The reaction mixture was stirred at room temperature for 6 hours and then at 35°C for 16 hours. Residue from solvent removal was purified by silica gel chromatography (hexane : ethyl acetate, 4 : 1) to give **60** (5.87 g, 79%). TLC: R_f=0.66 (hexane : ethyl acetate, 1 : 2). $[\alpha]_D^{22}$= - 37.2 (c1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.71 (d, J=2.8 Hz, 1 H, OH), 3.46 (m, 2 H, 2 H-6), 3.59 (m, 1 H, H-5), 3.80 (m, 1 H, H-4), 3.95 (m, 1 H, CH*H*CH=CH_2), 4.15 (dd, J=10.0, 8.5 Hz, 1 H, H-3), 4.16 (m, 1 H, CH*H*CH=CH_2), 4.25 (dd, J=10.0, 8.0 Hz, 1 H, H-2), 4.55 (d, J=12.0 Hz, CH*H*Ph), 4.84 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.85 (m, 1 H, CH*H*=CH), 5.02 (m, 1 H CH*H*=CH), 5.19 (d, J=8.0 Hz, 1 H, H-1), 5.59 (m, 1 H, CH_2=C*H*), 7.09 – 7.90 (m, 24 H, Ar-H). Anal. calcd for $C_{43}H_{39}NO_7$ (681.78): C, 75.75; H, 5.76; N, 2.04. Found: C, 75.37; H, 5.67; N, 2.04.

Example 53 Preparation of Compound **61**

To a mixture of sodium hydride (200 mg, 8.33 mmol), benzyl bromide (1.01 g, 0.70 ml, 8.35 mmol) and dry DMF (20 ml), a solution of **60** (3.80 g, 5.57 mmol) in dry DMF (20 ml) was added dropwise within 10 min. After stirring for 3 hours at room temperature, methanol (4 ml) was added and stirring was continued for 10 minutes further. Reaction was then poured into ice-cooled saturated sodium chloride solution (500 ml) and extracted with dichloromethane (200 ml x 3). Combined organic layers were dried with sodium sulfate and concentrated. The residue

was purified by flash chromatography (hexane : ethyl acetate, 4 : 1) to give **61** (2.45 g, 57%). TLC: R_f=0.67 (hexane : ethyl acetate, 2 : 1). $[\alpha]_D^{22}$= - 37.2 (c1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.32 (dd, J=10.0, 3.5 Hz, 1 H, H-6a), 3.62 (m, 1 H, H-5), 3.69 (dd, J=10.0, 1.0 Hz, 1 H, H-6b), 3.93 (m, 1 H, CH*H*CH=CH_2), 3.96 (m, 1 H, H-4), 4.25 (m, 1 H, CH*H*CH=CH_2), 4.27 (dd, J=10.5, 8.5 Hz, 1 H, H-2), 4.42 (dd, J=10.5, J=8.5 Hz, 1 H, H-3), 4.44 (d, J=10.0 Hz, 1 H, CH*H*Ph), 4.66 (d, J=12.0, Hz, 1 H, CH*H*Ph), 4.70 (d, J=10.0 Hz, 1 H, CH*H*Ph), 4.83 (m, 1 H, CH*H*=CH), 4.99 (d, J=12.0 Hz, 1 H, CH*H*Ph), 5.02 (m, 1 H, CH*H*=CH), 5.27 (d, J=8.5 Hz, 1 H, H-1), 5.59 (m, 1 H, CH_2=C*H*), 6.92 – 7.90 (m, 29 H, Ar-H). Anal. calcd for $C_{50}H_{45}NO_7$•$0.5H_2O$ (771.91): C, 76.90; H, 5.94; N, 1.79. Found: C, 76.72; H, 6.11; N, 1.78.

Example 54 Preparation of Compound **62**

Compound **61** (1.50 g, 1.94 mmol) was dissolved in acetic acid : water : allyl alcohol (8 : 2 : 1, 220 ml) and this solution was heated at 110°C for 1 h. The solvent was then removed in *vacuo* and the residue was purified by flash chromatography (hexane : ethyl acetate, 2.5 : 1 and then 2 : 1) to give **62** (0.90 g, 87%). TLC: R_f=0.33 (hexane : ethyl acetate, 2 : 1). $[\alpha]_D^{22}$= - 16.3 (c 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.90 (dd, J=6.0, 6.0 Hz, 1 H OH), 3.52 (m, 1 H, H-6a), 3.65 (dd, J=9.5, 8.5 Hz, 1 H, H-4), 3.75 (m, 1 H, H-5), 3.90 (m, 2 H, H-6b, CH*H*CH=CH_2), 4.20 (m, 2 H, H-3, CH*H*CH=CH_2), 4.28 (dd, J=10.0, 8.0 Hz, 1 H, H-2), 4.52 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.68 (d, J=10.5 Hz, 1 H, CH*H*Ph), 4.79 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.80 (m, 1 H, CH*H*=CH), 4.84 (d, J=10.5 Hz, 1 H, CH*H*Ph), 5.00 (m, 1 H, CH*H*=CH), 5.23 (d, J=8.0 Hz, 1 H, H-1), 5.55 (m, 1 H, CH_2=C*H*), 7.10 –7.85 (m, 14 H, Ar-H). Anal. calcd for $C_{31}H_{31}NO_7$.$0.7H_2O$ (529.59): C, 68.67; H, 6.02; N, 2.58. Found: C, 68.46; H, 5.93; N, 2.53.

Example 55 Preparation of Compound **63**

To the solution of **62** (0.90 g, 1.70 mmol) in 95% ethanol (60 ml) was added hydrazine monohydrate (3 ml). The mixture was heated under refluxing for 2 hours. Solvent was then removed in *vacuo* and the residue was purified by flash chromatography (1% methanol in dichloromethane) to give **63** (525 mg, 77%). TLC: R_f=0.28 (3% methanol in dichloromethane). $[\alpha]_D^{22}$= - 17.0 (c 0.5, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.75 (s, 3 H, NH_2, OH), 2.87 (dd, J=9.5, 8.0 Hz, 1 H, H-2), 3.35 (dd, J=9.5, 9.5 Hz, 1 H, H-4), 3.36 (m, 1 H, H-5), 3.57 (dd, J=9.5, 9.5 Hz, 1 H, H-3), 3.72 (dd, J=12.0, 4.0 Hz, 1 H, H-6a), 3.88 (dd, J=12.0, 2.5 Hz, 1 H, H-

6b), 4.24 (m, 1 H, CH*H*CH=CH$_2$), 4.36 (d, J=8.0 Hz, 1 H, H-1), 4.42 (m, 1 H, CH*H*CH=CH$_2$), 4.62 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.64 (d, J=1.0 Hz, 1 H, CH*H*Ph), 4.82 (d, J=11.0 Hz, 1 H, CH*H*Ph), 4.88 (d, J=11.5 Hz, 1 H, CH*H*Ph), 5.18 – 5.33 (m, 2 H, C*H*$_2$=CH), 5.97 (m, 1 H, CH$_2$=C*H*), 7.30 (m, 10 H, Ar-H).

Example 56 Preparation of Compound **64**

In a similar method as described for **40**, compound **63** (510 mg, 1.28 mmol) was coupled with **14** (870 mg, 1.92 mmol) in presence of DCC (659 mg, 3.20 mmol) to give **64** (853 mg, 80%) after silica gel chromatography (2 to 5% acetone in chloroform). TLC: R_f=0.38 (% methanol dichloromethane). $[\alpha]_D^{22}$= - 6.0 (c 1.0, chloroform). ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.89 (t, J=6.5 Hz, 6 H, 2 CH$_3$), 1.25 (br. s, 38 H, 19 CH$_2$), 1.59 (m, 4 H, 2 CH$_2$), 1.86 (t, J=7.0 Hz, 1 H, OH), 2.23 (t, J=7.5 Hz, 2 H, CH$_2$), 2.37 (dd, J=15.0, 5.5 Hz, 1 H, CH*H*), 2.48 (dd, J=15.0, 5.5 Hz, 1 H, CH*H*), 3.40 (m, 2 H, H-2, H-5), 3.52 (dd, J=9.5, 8.5 Hz, 1 H, H-4), 3.70 (m, 1 H, H-6a), 3.85 (m, 1 H, H-6b), 4.00 (dd, J=10.0, 8.5 Hz, 1 H, H-3), 4.14 (m, 1 H, CH*H*CH=CH$_2$), 4.26 (m, 1 H, CH*H*CH=CH$_2$), 4.59 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.63 (d, J=11.0 Hz, 1 H, CH*H*Ph), 4.82 (d, J=11.0 Hz, 1 H, CH*H*Ph), 4.83 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.96 (d, J=8.0 Hz, 1 H, H-1), 5.08 (m, 1 H, lipid-3-H), 5.13 (m, 1 H, CH*H*=CH), 5.23 (m, 1 H, CH*H*=CH), 5.88 (m, 1 H, C*H*=CH$_2$), 6.00 (d, J=8.0 Hz, 1 H, NH), 7.35 (m, 10 H, Ar-H). Anal. calcd for $C_{51}H_{81}NO_8$•0.7H_2O (836.20): C, 72.17; H, 9.78; N, 1.65. Found: C, 72.07; H, 9.81; N, 1.72.

Example 57 Preparation of Compound **65**

In a similar method as described for **44**, compound **65** was prepared using the imidate **43** (1.15 g, 1.12 mmol) and the glycosyl acceptor **64** (652 mg, 0.75 mmol) with BF$_3$ etherate (0.15 M in CH$_2$Cl$_2$, 3.5 ml). Silica gel chromatography (1 to 2% acetone in chloroform) yielded **65** (1.30 g, 83%). TLC: R_f=0.36 (6% acetone in chloroform). $[\alpha]_D^{22}$= - 18.6 (c 0.5, chloroform). ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.86 (t, J=6.5 Hz, 12 H, 4 CH$_3$), 1.22 (br. s, 76 H, 38 CH$_2$), 1.53 (m, 8 H, 4 CH$_2$), 2.15 (t, J=7.5 Hz, 2 H, CH$_2$), 2.20 (t, J=7.5 H, 2 H, CH$_2$), 2.32 (dd, J=14.0, 5.5 Hz, 1 H, CH*H*), 2.42 (dd, J=14.0, 6.0 Hz, 1 H, CH*H*), 2.47 (dd, J=15.0, 5.0 Hz, 1 H, CH*H*), 2.57 (dd, J=15.0, 7.0 Hz, 1 H, CH*H*), 3.34 – 4.21 (m, 12 H, H-2, H-3, H-4, H-5, 2 H-6, H-2', H-4', H-5', H-6'a, C*H*$_2$CH=CH$_2$), 4.30 (dd, J=10.0, 5.0 Hz, 1 H, H-6'b), 4.51 (d, J=8.5 Hz, 1 H, H-1'), 4.57 (m, 4 H, 2 CH*H*Ph, Cl$_3$CC*H*$_2$O), 4.78 (d, J=11.0 Hz, 1 H, CH*H*Ph), 4.85 (d, J=11.5 Hz, 1 H,

CH*H*Ph), 4.88 (d, J=8.0 Hz, 1 H, H-1), 5.00 – 5.25 (m, 5 H, H-3', 2 lipid-3-H, CH_2=CH), 5.45 (s, 1 H, C*H*Ph), 5.85 (m, 1 H, C*H*=CH_2), 6.00 (d, J=8.0 Hz, 1 H, NH), 7.30 (m, 15 H, Ar-H). Anal. calcd for $C_{95}H_{149}Cl_3N_2O_{17}$ (1697.58): C, 67.21; H, 8.85; N, 1.65. Found: C, 66.99; H, 8.96; N, 1.65.

Example 58 Preparation of Compound 67

(1) Compound 66: In the similar method as described for **50**, compound 65 (350 mg, 0.206 mmol) was treated with activated Zinc (9.0 g) and 80% acetic acid in ethyl acetate (500 ml) at room temperature to give **66** (314 mg, 100%), which was used directly in the next step.

(2) Compound 67: Compound **66** (304 mg, 0.20 mmol), **14** (191 mg, 0.42 mmol) and DCC (130 mg, 0.62 mmol) were dissolved in dry dichloromethane (10 ml). The mixture was stirred at room temperature for 24 hours and the solid was filtered off. The filtrate was concentrated and the residue was purified by flash chromatography (2% acetone in chloroform) to give **67** (220 mg, 56%). TLC: R_f=0.25 (5% acetone in chloroform). $[\alpha]_D^{22}$= - 16.0 (c 0.5, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J=6.5 Hz, 18 H, 6 CH_3), 1.23 (br. s, 114 H, 57 CH_2), 1.55 (m, 12 H, 6 CH_2), 2.13 – 2.48 (m, 10 H, 5 CH_2), 2.50 (dd, J=15.0, 5.5 Hz, 1 H, CH*H*), 2.59 (dd, J=15.0, 7.5 Hz, 1 H, CH*H*)), 3.38 – 4.24 (m, 12 H, H-2, H-3, H-4, H-5, 2 H-6, H-2', H-4', H-5', H-6'a, CH_2CH=CH_2), 4.30 (dd, J=10.5, 5.5 Hz, 1 H, H-6'b), 4.58 (d, J=11.0 Hz, 1 H, CH*H*Ph), 4.59 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.73 (d, J=8.5 Hz, 1 H, H-1'), 4.77 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.83 (d, J=8.0 Hz, 1 H, H-1), 4.85 (d, J=11.0 Hz, 1 H, CH*H*Ph), 4.99 – 5.30 (m, 6 H, H-3', CH_2=CH, 3 lipid-3-H), 5.48 (s, 1 H, C*H*Ph), 5.87 (m, 1 H, CH_2=C*H*), 5.91 (d, J=8.5 Hz, 1 H, NH), 6.07 (d, J=8.0 Hz, 1 H, NH), 7.35 (m, 15 H, Ar-H). Anal. calcd for $C_{120}H_{200}N_2O_{18}$ (1958.90): C, 73.58; H, 10.30; N, 1.43. Found: C, 73.40; H, 10.70; N, 1.39.

Example 59 Preparation of Compound **68**

In a similar way as described for **31**, compound 67 (194 mg, 0.10 mmol) was converted to **68** (130 mg, 67%). TL: R_f=0.22 (8% acetone in chloroform). $[\alpha]_D^{22}$= - 9.6 (c 0.5, chloroform). ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.89 (t, J=7.0 Hz, 18 H, 6 CH_3), 1.25 (br s, 114 H, 57 CH_2), 1.49 – 1.58 (m, 12 H, 6 CH_2), 2.21 (t, J=8.0 Hz, 2 H, CH_2), 2.26 (m, 3 H, CH_2, CH*H*), 2.27 (t, J=8.0 Hz, 2 H, CH_2), 2.33 (dd, J=14.0, 6.0 Hz, 1 H, CH*H*), 2.36 (dd, J=15.0, 6.5 Hz, 1 H, CH*H*), 2.43 (dd, J=15.0, 6.5 Hz, 1 H, CH*H*), 2.50 (dd, J=16.5, 6.50 Hz, 1 H, CH*H*), 2.53 (dd, J=16.5, 8.0 Hz, 1 H,

CH*H*), 3.28 (d, J=3.0 Hz, 1 H, OH), 3.43 (m, 2 H, H-4, H-5'), 3.54 (m, 2 H, H-2, H-6a), 3.62 (ddd, J=10.0, 9.0, 3.0 Hz, 1 H, H-4'), 3.71 (m, 3 H, H-5, 2 H-6'), 3.84 (m, 2 H, H-3, H-2'), 4.06 (dd, J=11.0, 2.5 Hz, 1 H, H-6b), 4.10 – 4.20 (m, 2 H, CH_2CH=CH_2), 4.52 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.58 (m, 4 H, H-1', 3 CH*H*Ph), 4.74 (d, J=10.5 Hz, 1 H, CH*H*Ph), 4.80 (d, J=8.0 Hz, 1 H, H-1), 4.83 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.95 (dd, J=10.0, 9.0 Hz, 1 H, H-3'), 5.02 (m, 1 H, lipid-3-H), 5.10 (m, 3 H, 2 lipid-3-H, CH*H*=CH), 5.22 (m, 1 H, CH*H*=CH), 5.77 (d, J=9.0 Hz, 1 H, NH), 5.85 (m, 1 H, CH_2=C*H*), 5.98 (d, J=8.0 Hz, 1 H, NH), 7.30 (m, 15 H, Ar-H). Anal. calcd for $C_{120}H_{202}N_2O_{18}$ (1960.92): C, 73.50; H, 10.38; N, 1.43. Found: C, 73.25; H, 10.95; N, 1.60.

Example 60 Preparation of Compound **69**

In a similar way as described for **32**, compound **68** (117 mg, 0.060 mmol) was converted to **69** (81 mg, 61%) which was purified by repeated flash chromatography (initially with 1 to 3% acetone in chloroform and then with toluene : acetone, from 15 : 1 to 12 : 1 and subsequently with hexane : acetone, from 6 : 1 to 5 : 1). TLC: R_f=0.46 (9% acetone in chloroform). $[\alpha]_D^{22}$= -4.8 (c 0.33, chloroform). ^1H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J=6.5 Hz, 18 H, 6 CH_3), 1.25 (br. s, 114 H, 57 CH_2), 1.45 – 1.55 (m, 12 H, 6 CH_2), 2.19 – 2.51 (m, 12 H, 6 CH_2), 3.45 – 4.23 (m, 12 H, H-2, H-3, H-4, H-5, 2 H-6, H-2', H-5', 2 H-6', CH_2CH=CH_2), 4.50 (m, 3 H, H-4', CH_2Ph), 4.58 (d, J=12.5 Hz, 2 H, 2 CH*H*Ph), 4.75 (d, J=11.0 Hz, 1 H, CH*H*Ph), 4.80 (d, J=8.0 Hz, 1 H, H-1), 4.88 (m, 5 H, 5 CH*H*Ph), 4.99 (d, J=8.0 Hz, 1 H, H-1'), 5.05 – 5.26 (m, 5 H, 3 lipid-3-H, CH_2=CH), 5.41 (dd, J=10.0, 9.0 Hz, 1 H, H-3'), 5.86 (m, 1 H, CH_2=C*H*), 5.93 (d, J=8.0 Hz, 1 H, NH), 6.09 (d, J=7.5 Hz, 1 H, NH), 7.30 (m, 25 H, Ar-H). Anal. calcd for $C_{134}H_{215}N_2O_{21}P$ (2221.15): C, 72.46; H, 9.76; N, 1.26. Found: C, 72.21; H, 9.92; N, 1.27.

Example 61 Preparation of Compound **70**

In a similar method as described for **33**, compound **69** (73 mg, 0.035 mmol) was converted to **70** (55 mg, 95%). TLC: R_f=0.35 (chloroform : methanol : water, 3 : 1 : 0.1). $[\alpha]_D^{22}$= + 6.0 (c 0.1, chloroform : methanol, 4 : 1). ES-MS calcd for $C_{99}H_{187}N_2O_{21}P$: 1771.3. Found (negative mode): 1770.3 (M-H), 1771.3 (M-H, isotopic peak).

Example 62 Preparation of Compound **71**

In a similar method as described for **42**, compound **65** (350 mg, 0.195 mmol) was converted to **71** (200 mg, 62%). TLC: R_f=0.30 (5% acetone in chloroform). $[\alpha]_D^{22}$= - 25.7 (c 0.83, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, J=6.5 Hz, 12 H, 4 CH_3), 1.25 (br. s, 76 H, 38 CH_2), 1.55 (m, 8 H, 4 CH_2), 1.70 (s, 1 H, OH), 2.17 (t J=7.0 Hz, 2 H, CH_2), 2.26 (t, J=7.0 Hz, 2 H, CH_2), 2.43 (m, 2 H, CH_2), 2.50 (dd, J=14.0, 5.5 Hz, 1 H, CH*H*), 2.60 (dd, J=15.0, 7.5 Hz, 1 H, CH*H*), 3.40 – 3.90 (m, 9 H, H-2, H-3, H-4, H-5, H-6a, H-2', H-4', H-5', H-6'a), 4.13 (br. d, J=10.0 Hz, 1 H, H-6b), 4.34 (dd, J=10.0, 5.0 Hz, 1 H, H-6'b), 4.51, 4.52 (2 d, J=8.5 Hz, each 1 H, H-1, H-1'), 4.60 (d, J=12.5 Hz, 1 H, CH*H*Ph), 4.66 (m, 3 H, CH*H*Ph, Cl_3CCH_2O), 4.90 (d, J=12.5 Hz, 1 H, CH*H*Ph), 4.98 (d, J=11.5 Hz, 1 H, CH*H*Ph), 5.04 – 5.25 (m, 3 H, H-3', 2 lipid-3-H), 5.49 (s, 1 H, C*H*Ph), 6.02 (d, J=5.0 Hz, 1 H, NH), 7.40 (m, 15 H, Ar-H). Anal. calcd for $C_{92}H_{145}Cl_3N_2O_{17}$•$0.8H_2O$ (1657.52): C, 66.09; H, 8.84; N, 1.67. Found: C, 66.06; H, 8.84; N, 1.64.

Example 63 Preparation of Compound **72**

In a similar method as described for **28**, compound **71** (670 mg, 0.405 mmol) was coupled with **17** (270 mg, 0.81 mmol) in presence of DCC (208 mg, 1.01 mmol) and DMAP (25 mg, 0.20 mmol). The reaction was completed in 72 h. Silica gel chromatography (dichloromethane : hexane : acetone, 2 : 1 : 3%; and 1% methanol in dichloromethane) afforded **72** (570 mg, 71%). TLC: R_f=0.60 (dichloromethane : hexane : acetone, 10 : 5 : 1). $[\alpha]_D^{22}$= - 20.0 (c 1.0, chloroform). ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.88 (t, J=7.0 Hz, 15 H, 5 CH_3), 1.25 (m, 74 H, 37 CH_2), 1.53 (m, 10 H, 5 CH_2), 2.17 (t, J=7.5 Hz, 2 H, CH_2), 2.22 (dd, J=15.0, 6.0 Hz, 1 H, CH*H*), 2.24 (t, J=7.5 Hz, 2 H, CH_2), 2.34 (dd, J=15.0, 6.5 Hz, 1 H, CH*H*), 2.45 (dd, J=16.0, 5.0 Hz, 1 H, CH*H*), 2.50 (dd, J=15.5, 5.5 Hz, 1 H, CH*H*), 2.55 (dd, J=16.0, 7.5 Hz, 1 H, CH*H*), 2.59 (dd, J=15.5, 7.5 Hz, 1 H, CH*H*), 3.38 (ddd, J=10.0, 10.0, 5.0 Hz, 1 H, H-5'), 3.56 (m, 2 H, H-4, H-5), 3.62 (m, 1 H, H-2), 3.64 (dd, J=10.0, 10.0 Hz, 1 H, H-4'), 3.69 (dd, J=11.0, 5.0 Hz, 1 H, H-6a), 3.76 (dd, J=10.0, 10,0 Hz, 1 H, H-6'a), 3.83 (m, 1 H, lipid-3-H), 3.95 (m, 1 H, H-2'), 4.05 (br. d, J=11.0 Hz, 1 H, H-6b), 4.32 (dd, J=10.0, 5.0 Hz, 1 H, H-6'b), 4.45 (d, J=11.0 Hz, 1 H, CH*H*Ph), 4.48 (d, J=11.0 Hz, 2 H, 2 CH*H*Ph), 4.51 (d, J=8.0 Hz, 1 H, H-1), 4.59 (d, J=8.0 Hz, 1 H, H-1'), 4.60 – 4.67 (m, 4 H, 2 CH*H*Ph, Cl_3CCH_2O), 4.85 (d, J=12.0 Hz, 1 H, CH*H*Ph), 5.01 (m, 1 H, lipid-3-H), 5.12 (d, J=9.0 Hz, 1 H, NH), 5.19 (m, 4 H, H-3, H-3', 2 lipid-3-H), 5.48 (s, 1 H, C*H*Ph), 5.71

(d, J=8.0 Hz, 1 H, NH), 7.20 – 7.45 (m, 20 H, Ar-H). Anal. calcd for $C_{113}H_{177}Cl_3N_2O_{19}$ (1974.00): C, 68.76; H, 9.04; N, 1.42. Found: C, 68.68; H, 9.10; N, 1.39.

Example 64 Preparation of Compound **74**

(1) Compound **73**: In the similar method as described for **50**, compound 72 (550 mg, 0.279 mmol) was treated with activated Zinc (9.0 g) and 80% acetic acid in ethyl acetate (500 mg) at room temperature to give **73** (500 mg, 100%). TLC: R_f=0.12 (2% methanol in dichloromethane).

(2) Compound **74**: Compound **73** (270 mg, 0.15 mmol), **5** (211 mg, 0.45 mmol) and DCC (139 mg, 0.68 mmol) were dissolved in dry CH_2Cl_2 : DMF (4 : 1, 15 ml) and the mixture was stirred at room temperature for 24 h. The solvent was removed and the residue was purified by silica gel chromatography (3 to 7% acetone in chloroform) to give **74** (220 mg, 65%). TLC: R_f=0.15 (8% acetone in chloroform). $[\alpha]_D^{22}$= - 20.0 (c 0.5, chloroform). ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.90 (m, 21 H, 7 CH_3), 1.25 (m, 126 H, 63 CH_2), 1.50 (m, 14 H, 7 CH_2), 2.13 (m, 2 H, CH_2), 2.16 – 2.25 (m 5 H, 2 CH_2, CH*H*), 2.31 (dd, J=15.5, 7.0 Hz, 1 H, CH*H*), 2.36 (dd, J=15.0, 7.0 Hz, 1 H, CH*H*), 2.45 (dd, J=16.0, 5.0 Hz, 1 H, CH*H*), 2.50 (m, 2 H, 2 CH*H*), 2.55 (dd, J=15.5, 5.5 Hz, 1 H, CH*H*), 2.60 (dd, J=15.0, 7.5 Hz, 1 H, CH*H*), 3.17 (m, 2 H, NCH_2), 3.42 (ddd, J=10.0, 10.0, 5.0 Hz, 1 H, H-5'), 3.52 (dd, J=10.0, 9.0 Hz, 1 H, H-4), 3.61 (dd, J=10.0, 10.0 Hz, 1 H, H-4'), 3.64 (m, 2 H, H-5, H-2'), 3.73 (m, 1 H, H-6a), 3.75 (dd, J=10.0, 10.0 Hz, 1 H, H-6'a), 3.82 (m, 1 H, lipid-3-H), 4.06 (m, 2 H, H-2. H-6b), 4.33 (dd, J=10.0, 5.0 Hz, 1 H, H-6'b), 4.44 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.46 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.47 (m, 1 H, Asp-α-H), 4.49 (d, J=11.5 Hz, 1 H, CH*H*Ph), 4.57 (d, J=11.5 H, 1 H, CH*H*Ph), 4.67 (d, J=12.5 Hz, 1 H, CH*H*Ph), 4.73 (d, J=8.0 Hz, 1 H, H-1), 4.85 (d, J=12.5 Hz, 1 H, CH*H*Ph), 4.89 (d, J=8.5 Hz, 1 H, H-1'), 5.06 (m, 1 H, lipid-3-H), 5.13 (m, 2 H, 2 lipid-3-H), 5.21 (dd, J=10.0, 9.0 Hz, 1 H, H-3), 5.38 (dd, J=10.0, 10.0 Hz, 1 H, H-3'), 5.47 (s, 1 H, C*H*Ph), 5.97 (d, J=9.0 Hz, 1 H, NH), 6.46 (d, J=8.0 Hz, 1 H, NH), 7.05 (t, J=5.0 Hz, 1 H, NH), 7.10 (d, J=8.0 Hz, 1 H, NH), 7.22 – 7.45 (m, 20 H, Ar-H). Anal. calcd for $C_{137}H_{226}N_4O_{20}$ (2249.31): C, 73.15; H, 10.13; N, 2.49. Found: C, 73.00; H, 10.51; N, 2.41.

Example 65 Preparation of Compound **75**

In a similar way as described for **31**, compound 74 (140 mg, 0.062 mmol) was treated with sodium cyanoborohydride (780 mg, 12.4 mmol) and HCl (g) / Et_2O in dry THF (25 ml) at room

temperature to give **75** (90 mg, 64%). TLC: R_f=0.17 (10% acetone in chloroform). $[\alpha]_D^{22}$= - 16.0 (c 0.5, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J=6.5 Hz, 21 H, 7 CH_3), 1.25 (br. s, 126 H, 63 CH_2), 1.40 – 1.60 (m, 14 H, 7 CH_2), 2.15 – 2.60 (m, 14 H, 7 CH_2), 3.15 (m, 2 H, NCH_2), 3.33 (d, J=3.0 Hz, 1 H, OH), 3.43 – 4.12 (m, 11 H, H-2, H-4, H-5, 2 H-6, H-2', H-4', H-5', 2 H-6', lipid-3-H), 4.41 – 4.59 (m, 6 H, Asp-α-H, 5 CH*H*Ph), 4.61 (d, J=11.0 Hz, 1 H, CH*H*Ph), 4.65 (d, J=12.0 Hz, 1 H, CH*H*Ph), 4.70 (d, J=8.5 Hz, 1 H, H-1), 4.77 (d, J=8.0 Hz, 1 H, H-1'), 4.85 (d, J=12.0 Hz, 1 H, CH*H*Ph), 5.04 – 5.21 (m, 4 H, H-3, H-3', 2 lipid-3-H), 5.96 (d, J=9.0 Hz, 1 H, NH), 6.31 (d, J=8.0 Hz, 1 H, NH), 7.08 (t, J=5.0 Hz, 1 H, NH), 7.15 – 7.30 (m, 21 H, NH, Ar-H). Anal. calcd for $C_{137}H_{228}N_4O_{20}$ (2251.32): C, 73.09; H, 10.21; N, 2.48. Found: C, 73.31; H, 10.77; N, 2.41.

Example 66 Preparation of Compound **76**

In a similar way as described for **32**, compound **75** (120 mg, 0.053 mmol) was converted to **76** (81 mg, 60%) which was purified by repeated flash chromatography (initially with 3% acetone in chloroform and then with toluene : acetone from 10 : 1 to 8 : 1). TLC: R_f=0.30 (9% acetone in chloroform). $[\alpha]_D^{22}$= - 10.9 (c 0.33, chloroform). ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.90 (t, J=7.0 Hz, 21 H, 7 CH_3), 1.25 (br. s, 126 H, 63 CH_2), 1.45 – 1.60 (m, 14 H, 7 CH_2), 2.15 – 2.55 (m, 14 H, 7 CH_2), 3.16 (m, 2 H, NCH_2), 3.51 – 4.10 (m, 10 H, H-2, H-4, H-5, 2 H-6, H-2', H-5', 2 H-6', lipid-3-H), 4.38 – 4.50 (m, 7 H, H-4', 6 CH*H*Ph), 4.55 (d, J=11.0 Hz, 1 H, CH*H*Ph), 4.65 (d, J=12.5 Hz, 1 H, CH*H*Ph), 4.70 (d, J=8.0 Hz, 1 H, H-1), 4.84 (d, J=12.5 Hz, 1 H, CH*H*Ph), 4.87 – 4.92 (m, 5 H, Asp-α-H, H-1', 3 CH*H*Ph), 5.07 (m, 2 H, 2 lipid-3-H), 5.18 (dd, J=10.0, 10.0 Hz, 1 H, H-3), 5.31 (dd, J=10.0, 10.0 Hz, 1 H, H-3'), 5.93 (d, J=9.0 Hz, 1 H, NH), 6.51 (d, J=8.0 Hz, 1 H, NH), 7.03 (t, J=5.5 Hz, 1 H, NH), 7.09 (d, J=7.0 Hz, 1 H, NH), 7.16 – 7.33 (m, 30 H, Ar-H). Anal. calcd for $C_{151}H_{241}N_4O_{23}P$ (2511.55): C, 72.21; H, 9.67; N, 2.23. Found: C, 72.13; H, 9.82; N, 2.17.

Example 67 Preparation of Compound **77**

In the same way as described for **33**, compound **76** (70 mg, 0.03 mmol) was converted to **77** (42.5 mg, 78%). TLC: R_f=0.37 (chloroform : methanol : water, 3 : 1 : 0.1), $[\alpha]_D^{22}$= - 11.0 (c 0.1, chloroform : methanol, 4 : 1). ES-MS calcd for $C_{109}H_{205}N_4O_{23}P$: 1969.5. Found (negative mode): 1968.5 (M-H), 1969.5 (M-H, isotopic peak).

Example 68 Preparation of Compound **78**

The lipid acid **17** (1.5g, 4.5 mmol), the sugar compound **27** (3.18g, 7.8 mmol), EDCI (1.3g, 6.8 mmol) and DMAP (0.275g, 2.2 mmol) were taken in anhydrous dichloromethane (40 ml) and stirred for 3 hours at room temperature under nitrogen atmosphere. TLC indicated the completion of the reaction. The solvent was removed in *vacuo* and the colourless residue was purified silica gel chromatography (10% ethyl acetate in hexane) to give **78** (2.4 g, 70%). TLC: R_f=0.54 (hexane : ethyl acetate, 3 : 1). $[\alpha]_D^{20}$= +69.1 (c 0.53, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 3 H, CH_3), 1.25 (br s, 18 H), 1.32 (s, 3 H, CH_3), 1.43 (s, 3 H, CH_3), 1.51 (m, 2 H), 2.42 (dd, J=15.5, 5.5 Hz, 1 H), 2.68 (dd, J=15.5, 6.5 Hz, 1 H), 3.71 – 3.86 (m, 5 H), 4.02 (ddd, J=10.0, 10.0, 3.5 Hz, 1 H, H-2), 4.45 (d, J=11.5 Hz, 1 H), 4.49 (d, J=12.0 Hz, 1 H), 4.55 (d, J=12.0 Hz, 1 H), 4.58 (d, J=11.5 Hz, 1 H), 4.69 (d, J=12.0 Hz, 1 H), 4.71 (d, J=12.0 Hz, 1 H), 4.92 (d, J=3.5 Hz, 1 H, H-1), 5.24 (dd, J=10.0, 10.0 Hz, 1 H, H-3), 5.32 (d, J=10.0 Hz, 1 H, NH), 7.30 (m, 10 H, Ar-H).

Example 69 Preparation of Compound **79**

Compound **78** (2.4g) and 80% acetic acid in ethyl acetate (50 ml) was stirred at 35°C for 2 hours. The solvent was then removed under reduced pressure and co-evaporated with toluene. The colorless residue obtained was purified by silica gel chromatography (10% acetone in toluene) to give **79** (2.1 g, 85%). TLC: R_f=0.25 (toluene : acetone, 4 : 1). $[\alpha]_D^{20}$= +54.6 (c 2.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, CH_3), 1.25 (br s, 18 H, 9 CH_2), 1.51 (m, 2 H, CH_2), 1.99 (br s, 1 H, OH), 2.49 (dd, J=14.5, 4.5 Hz, 1 H), 2.61 (dd, J=14.5, 7.5 Hz, 1 H), 3.01 (br s, 1 H, OH), 3.62 – 3.95 (m, 6 H), 4.98 (d, J=12.0 Hz, 1 H), 4.50 (d, J=12.0 Hz, 1 H), 4.53 (d, J=12.0 Hz, 1 H), 4.62 (d, J=12.0 Hz, 1 H), 4.67 (d, J=12.0 Hz, 1 H), 4.94 (d, J=3.5 Hz, 1 H, H-1), 5.15 (dd, J=10.0, 9.0 Hz, 1 H, H-3), 5.29 (d, J=9.0 Hz, 1 H, NH). 7.30 (m, 10 H, Ar-H).

Example 70 Preparation of Compound **80**

In a similar method as described for **46,** compound **41** (1.45 g, 1.60 mmol) was treated with sodium cyanoborohydride (1.0 g, 15.96 mmol) and freshly saturated solution of diethyl ether with hydrogen chloride gas to give **80** (1.23 g, 85%) after flash silica gel chromatography (initially with hexane : ethyl acetate, 5 : 1 and then 4 : 1). TLC: R_f=0.20 (hexane : ethyl acetate, 4

: 1). $[\alpha]_D^{20}$ = +47.5 (c 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.25 (br s, 38 H, 19 CH_2), 1.50 (m, 4 H, 2 CH_2), 2.28 (t, J=7.5 Hz, 2 H, CH_2), 2.48 (dd, J=14.0, 4.0 Hz, 1 H), 2.58 (dd, J=14.0, 7.5 Hz, 1 H), 3.27 (d, J=3.5 Hz, 1 H, OH), 3.70 – 3.86 (m, 4 H), 3.92 – 4.03 (m, 2 H), 4.58 (d, J=12.0 Hz, 1 H), 4.64 (d, J=12.0 Hz, 1 H), 4.66 (d, J=12.0 Hz, 1 H), 4.76 (d, J=12.0 Hz, 1 H), 4.92 (d, J=3.5 Hz, 1 H, H-1), 5.13 (m, 2 H), 5.19- 5.31 (m, 2 H, CH_2=CH), 5.40 (d, J=9.5 Hz, 1 H, NH), 5.88 (m, 1 H, CH_2=C*H*), 7.30 (m, 5 H, Ar-H). ES-MS calcd for $C_{47}H_{76}Cl_3NO_{10}$: 919.5. Found: 920.8 (M+H).

Example 71 Preparation of Compound **81**

In a similar way as described for **47**, compound **80** (1.20 g, 1.30 mmol) was treated with 1*H* tetrazole and dibenzyl diisopropylphosphoramidite (900 mg, 0.875 ml, 2.61 mmol) in dry dichloromethane (12 ml) and followed by with m-CPBA (1.63 g, 55%, 5.22 mmol) to give **81** (1.33 g, 86%) after flash silica gel chromatography (initially hexane : ethyl acetate, 4 : 1 and then 3 : 1). TLC: R_f=0.31 (hexane : ethyl acetate, 3 : 1). $[\alpha]_D^{20}$ = +35.0 (c 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.24 (br s, 38 H, 19 CH_2), 1.50 (m, 4 H, 2 CH_2), 2.17 (t, J=7.0 Hz, 2 H, CH_2), 2.41 (dd, J=16.5, 5.5 Hz, 1 H), 2.51 (dd, J=16.5, 7.5 Hz, 1 H), 3.66 (dd, J=11.0, 4.5 Hz, 1 H), 3.74 (dd, J=11.0, 2.0 Hz, 1 H), 3.91 (m, 1 H), 4.00 (m, 2 H), 4.20 (m, 1 H), 4.44 (d, J=12.0 Hz, 1 H), 4.53 (m, 1 H, H-4), 4.54 (d, J=12.0, 1 H), 4.63 (d, J=12.0, 1 H), 4.88 – 4.95 (m, 5 H), 5.11 (m, 1 H), 5.20 – 5.32 (m, 2 H, CH_2=CH), 5.35 (dd, J=10.5, 9.0 Hz, 1 H, H-3), 5.41 (d, J=9.5 Hz, 1 H, NH), 5.88 (m, 1 H, CH_2=C*H*), 7.30 (m, 15 H, Ar-H). ES-MS calcd for $C_{61}H_{89}Cl_3NO_{13}P$: 1179.6, Found: 1181.0 (M+H).

Example 72 Preparation of Compound **82**

In a similar way as described for **42**, compound **81** (1.30 g, 1.10 mmol) was dissolved in dry THF (10 ml) and treated with [bis(methyldiphenylphosphine](1.5-cyclooctadiene) iridium (I) hexafluorophosphate (14 mg, 0.0165 mmol), followed by the treatment with water (0.5 ml) and N-succinimide (NBS, 294 mg, 1.62 mmol) to give **82** (950 mg, 76%). TLC: R_f=0.31 (ethyl acetate : hexane, 1 : 2). $[\alpha]_D^{20}$ = +17.5 (c 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.24 (br s, 38 H, 19 CH_2), 1.50 (m, 4 H, 2 CH_2), 2.18 (t, J=7.0 Hz, 2 H, CH_2), 2.39 (m, 2 H, CH_2), 3.59 (dd, J=11.0, 6.0 Hz, 1 H), 3.71 (dd, J=11.0, 1.5 Hz, 1 H), 3.94 (m, 1 H), 4.16 (m, 1 H), 4.40 (m, 3 H), 4.49 (d, J=12.0 Hz, 1 H), 4.65 (d, J=12.0 Hz, 1 H), 4.72

(d, J=12.0 Hz, 1 H), 4.90 (m, 4 H), 5.09 (m, 1 H), 5.39 (t, J=3.5 Hz, 1 H, H-1), 5.37 (dd, J=10.0, 9.5 Hz, 1 H, H-3), 5.70 (d, J=9.5 Hz, 1 H, NH), 7.30 (m, 15 H, Ar-H). ES-MS calcd for $C_{58}H_{85}Cl_3NO_{13}P$: 1139.5. Found: 1141.0 (M+H).

Example 73 Preparation of Compound **83**

In a similar method as described for **43**, compound **82** (920 mg, 0.81 mmol) was treated with trichloroacetonitrile (2 ml) and DBU (4 drops). Purification by flash silica gel chromatography (hexane : ethyl acetate, 4 : 1, 3.5 : 1 and 3 : 1, with 0.5% of triethyl amine) afforded **83** (700 mg, 68%). TLC: R_f=0.36 (hexane : ethyl acetate, 3 : 1). $[\alpha]_D^{20}$ = +12.5 (c 0.4, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.24 (br s, 38 H, 19 CH_2), 1.50 (m, 4 H, 2 CH_2), 2.19 (t, J=7.0 Hz, 2 H, CH_2), 2.46 (m, 2 H, CH_2), 3.71 (m, 2 H), 4.04 (m, 1 H). 4.15 (ddd, J=1.0, 8.5, 3.5 Hz, 1 H, H-2), 4.43 (d, J=12.0 Hz, 1 H), 4.52 (d, J=12.0 Hz, 1 H), 4.61 (d, J=12.0 Hz, 1 H), 4.71 (ddd, J=9.5, 9.5, 9.5 Hz, 1 H, H-4), 4.77 (d, J=12.0 Hz, 1 H). 4.94 (m, 4 H), 5.12 (m, 1 H), 4.39 (dd, J=10.0, 9.5 Hz, 1 H, H-3), 5.65 (d, J=8.5 Hz, 1 H, NH), 6.47 (d, J=3.5 Hz, 1 H, H-1), 7.32 (m, 15 H, Ar-H), 8.72 (s, 1 H, NH). ES-MS calcd for $C_{60}H_{85}Cl_6N_2O_{13}P$: 1282.4. Found: 1284.0 (M+H).

Example 74 Preparation of Compound **84**

In a similar way as described for **44**, the imidate **83** (190 mg, 0.148 mmol) and **79** (112 mg, 0.148 mmol) were treated with trifluoroboron diethyl etherate in dichloromethane solution (0.1 M, 0.4 ml) to give **84** (172 mg, 66%). TLC: R_f=0.25 (hexane : ethyl acetate, 2.5 : 1). $[\alpha]_D^{20}$ = +17.5 (c 2.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.88 (t, J=6.5 Hz, 9 H, 3 CH_3), 1.24 (br s, 56 H, 28 CH_2), 1.42 – 1.62 (m, 6 H, 3 CH_2), 2.23 (m, 2 H, CH_2), 2.38 (dd, J=15.0, 8.0 Hz, 1 H), 2.46 (dd, J=15.0, 4.0 Hz, 1 H), 2.47 (dd, J=15.0, 5.0 Hz, 1 H), 2.63 (dd, J=15.0, 7.5 Hz, 1 H), 3.00 (s, 1 H, OH), 3.48 (m, 1 H, lipid-3-H), 3.61 (dd, J=11.0, 5.5 Hz, 1 H), 3.65 (m, 2 H), 3.78 (dd, J=11.0, 2.0 Hz, 1 H), 3.83 (m, 3 H), 3.94 (ddd, J=10.0, 10.0, 4.0 Hz, 1 H, H-2), 4.06 (br d, J=10.0 Hz, 1 H), 4.42 (ddd, J=9.5, 9.5, 9.5 Hz, 1 H, H-4'), 4.45 (d, J=12.0 Hz, 1 H), 4.50 (m, 4 H), 4.52 (d, J=12.0 Hz, 1 H), 4.57 (d, J=12.0 Hz, 1 H), 4.66 (d, J=12.0 Hz, 1 H), 4.72 (m, 2 H), 4.90 (m, 6 H), 5.15 (dd, J=10.0, 10.0 Hz, 1 H, H-3), 5.19 (m, 1 H, lipid-3-H), 5.25 (d, J=10.0 Hz, 1 H, NH), 5.38 (dd, J=10.0, 10.0 Hz, 1 H, H-3'), 5.61 (d, J=8.0 Hz, 1 H, NH), 7.30 (m, 25 H, Ar-H). The assignment was based on ^{1}H-^{1}H COSY spectrum.

For structural proof, compound **84** was treated with acetic anhydride and pyridine to give its mono-acetate. TLC: R_f=0.30 (toluene : acetone, 8 : 1). $[\alpha]_D^{20}$ = +21.4 (c 0.4, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J=6.5 Hz, 9 H, 3 CH_3), 1.23 (br s, 56 H, 28 CH_2), 1.50 (m, 6 H, 3 CH_2), 1.80 (s, 3 H, CH_3), 2.18 (t, J=7.0 Hz, 2 H, CH_2), 2.44 (m, 3 H), 2.53 (dd, J=16.0, 7.0 Hz, 1 H), 3.45 (m, 1 H), 3.61 (m, 3 H), 3.80 (m, 2 H), 3.97 (m, 3 H), 4.42 (m, 1 H), 4.49 (m, 6 H), 4.58 – 4.75 (m, 5 H), 4.89 (m, 2 H), 4.91 (m, 2 H), 4.96 (d, J=3.5 Hz, 1 H, H-1), 5.05 (dd, J=10.0, 10.0 Hz, 1 H, H-4), 5.24 (d, J=10.0 Hz, 1 H, NH), 5.32 (dd, J=10.0, 10.0 Hz, 2 H, H-3, H-3'), 5.70 (d, J=8.0 Hz, 1 H, NH), 7.30 (m, 25 H, Ar-H).

Example 75 Preparation of Compound **85**

Compound **84** (30 mg, 0.017 mmol) was dissolved in acetic acid (10 ml) and treated with zinc (1.0 g) at room temperature for 30 min. The solid was filtered off and washed with dichloromethane (50 ml). The solvent was removed and the residue purified by flash silica gel chromatography (1% to 3% methanol in dichloromethane) to give the free amine compound (20 mg, 78%).

The solution of the above free amine compound (19 mg, 0.012 mmol), **14** (22.5 mg, 0.049 mmol) and DCC (10.2 mg, 0.049 mmol) in dry dichloromethane (3 ml) was stirred at room temperature for 20 h. The solvent was removed and the residue purified by repeated flash silica gel chromatography (initially with 0.5% to 2% methanol in dichloromethane and then hexane : acetone, 6 : 1) to give **85** (16 mg, 54%). TLC: R_f=0.43 (4% methanol in dichloromethane). $[\alpha]_D^{20}$ = +30.0 (c 0.25, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.90 (m, 21 H, 7 CH_3), 1.25 (m, 132 H, 66 CH_2), 1.50 (m, 14 H, 7 CH_2), 2.18 (dd, J=8.5, 3.5 Hz, 1 H), 2.20 –2.37 (m, 9 H), 2.41 (dd, J=14.5, 7.5 Hz, 1 H), 2.44 (dd, J=15.5, 4.5 Hz, 1 H), 2.48 (dd, J=15.5, 3.5 Hz, 1 H), 2.63 (dd, J=15.5, 7.0 Hz, 1 H), 3.58 – 3.63 (m, 2 H), 3.67 (m, 1 H), 3.72 – 3.79 (m, 4 H), 3.81 – 3.86 (m, 2 H), 4.00 (dd, J=11.0, 2.5 Hz, 1 H), 4.24 (ddd, J=10.5, 9.0, 3.5 Hz, 1 H, H-2), 4.38 (ddd, J=9.0, 9.0, 9.0 Hz, 1 H, H-4'), 4.44 (d, J=12.0, 1 H), 4.44 (d, J=11.5 Hz, 1 H), 4.45 (d, J=12.0 Hz, 1 H), 4.51 (d, J=12.0 Hz, 1 H), 4.54 (d, J=11.5 Hz, 1 H), 4.70 (d, J=12.0 Hz, 1 H), 4.88 (m, 5 H), 4.99 (d, J=8.0 Hz, 1 H, H-1'), 5.05 (m, 1 H), 5.13 (m, 2 H), 5.18 (dd, J=10.5, 9.0 Hz, 1 H, H-3), 5.34 (dd, J=10.5, 9.0 Hz, 1 H, H-3'), 5.92 (d, J=9.0 Hz, 1 H, NH), 6.27 (d, J=7.5 Hz, 1 H, NH), 7.30 (m, 25 H, Ar-H). ES-MS calcd for $C_{145}H_{237}N_2O_{23}P$: 2405.7. Found: 1204.4 ($M+2H^+$).

Example 76 Preparation of Compound **86**

In a similar method as described for **48**, compound **85** (12.0 mg, 0.005 mmol) was converted to **86** (9.5 mg, 97%). R_f=0.46 (chloroform : methanol : water, 3 : 1 : 1). $[\alpha]_D^{20}$ = - 6.6 (c 0.1, chloroform / methanol, 4 : 1). ES-MS calcd for $C_{110}H_{207}N_2O_{23}P$: 1955.5. Found: 1954.5 (M-H, negative mode).

Example 77 Preparation of Compound **87**

In a similar method as described for **78,** lipid acid **20** (547.9 mg, 1.33 mmol) was treated with EDCI (286.82 mg, 1.33 mmol), DMAP (85.36 mg, 1.15 mmol) and **28** (706.14 mg, 1.46 mmol) in dry CH_2Cl_2 (14.0 ml) to give **87** (1.08 g, 92%) after flash chromatography (ethyl acetate : hexane, 1 : 9). TLC: R_f=0.31 (ethyl acetate : hexane, 1 : 7). $[\alpha]_D^{20}$ = + 33.1 (c 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.25 (m, 36 H), 1.40 (m, 4 H), 2.35 (dd, J=15.0, 6.0 Hz, 1 H), 2.60 (dd, J=15.0, 6.5 Hz, 1 H), 3.28 (m, 1 H), 3.41 (m, 1 H), 3.63 (m, 1 H), 3.72 (dd, J=9.5, 9.5 Hz, 1 H), 3.79 (dd, J=10.0, 10.0 Hz, 1 H), 3.91 – 4.10 (m, 3 H), 4.23 (m, 1 H), 4.23 (m, 1 H), 4.30 (dd, J=10.0, 4.5 Hz, 1 H), 4.67 (d, J=12.0 Hz, 1 H), 4.76 (d, J=12.0 Hz, 1 H), 4.94 (d, J=3.5 Hz, 1 H, H-1), 5.23 – 5.39 (m, 3 H), 5.41 (dd, J=10.0, 10.0 Hz, 1 H, H-3), 5.52 (s, 1 H), 5.90 (m, 1 H), 7.34 (m, 3 H), 7.44 (m, 2 H).

Example 78 Preparation of compound **88**

Acetonitrile (140 ml) was added to **87** (1.655 g; 1.863 mmol) and cooled 0°C. To this suspension was added $BH_3.Me_2NH$ (550.8 mg, 9.49 mmol), stirred for three minutes and then treated slowly with $BF_3.OEt_2$ (1.2 ml). After the addition, the reaction was allowed to stir at 0°C and then 45 minutes at room temperature. The clear reaction mixture was poured into a separate funnel containing saturated $NaHCO_3$ (50.0 ml), the water layer was then separated and washed with ethyl acetate (150 ml). Combined organic layers were concentrated to dryness and its remainder was purified by flash silica gel chromatography (ethyl acetate : hexane, 1 : 7) to give compound **88** (1.25g, 72%). TLC: R_f=0.14 (hexane : ethyl acetate, 7 : 1). $[\alpha]_D^{20}$ = + 35.6 (c 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (m, 6 H, 2 CH_3), 1.25 (m, 36 H), 1.50 – 1.60 (m, 4 H), 2.45 (dd, J=15.0, 5.0 Hz, 1 H), 2.67 (dd, J=15.0, 7.0 Hz, 1 H), 3,04 (d, J=2.5 Hz, 1 H), 3.42 (m, 2 H), 3.66 – 3.87 (m, 6 H), 4.00 (m, 2 H), 4.20 (m, 1 H), 4.58 (d, J=12.0 Hz, 1 H), 4.64

(d, J=12.0 Hz, 1 H), 4.68 (d, J=12.0 Hz, 1 H), 4.73 (d, J=12.0 Hz, 1 H), 4.94 (d, J=3.5 Hz, 1 H, H-1), 5.12 – 5.33 (m, 4 H), 5.90 (m, 1 H), 7.30 (m, 5 H).

Example 79 Preparation of Compound **89**

In a similar method as described for **32**, compound **88** (1.21 g; 1.364 mmol) was treated with 1*H*-tetrazole (288 mg; 4.11 mmol), dibenzyl diisopropylphosphoramidite (916 μl) and then with m-CPBA (942 mg, 5.456 mmol). Flash silica gel column (acetone : hexane, 1 : 7) provided compound **89** in 72% yield. TLC: R_f=0.53 (hexane / ethyl acetate, 4 :1). $[\alpha]_D^{20}$ = + 37.6 (c 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.25 (m, 36 H), 1.40 (m, 4 H), 2.31 (dd, J=16.0, 6.5 Hz, 1 H), 2.53 (dd, J=16.0, 6.0 Hz, 1 H), 3.19 (m, 1 H), 3.33 (m, 1 H), 3.55 (m, 1 H), 3.72 (m, 2 H), 3.90 – 4.04 (m, 3 H), 4.20 (m, 1 H), 4.46 (d, J=12.0 Hz, 1 H), 4.53 - 4.63 (m, 3 H), 4.81 (d, J=12.0 Hz, 1 H), 4.92 (m, 5 H), 5.21 – 5.33 (m, 3 H), 5.39 (dd, J=10.0, 10.0 Hz, 1 H, H-3), 5.90 (m, 1 H), 7.30 (m, 15 H, Ar-H).

Example 80 Preparation of Compound **90**

In a similar method as described for **42**, compound **89** (1.13 g, 0.98 mmol) was treated with saturated solution of [bis(methyldiphenylphosphine)](1,5-cyclooctadiene) iridium(I) hexafluorophosphate (25.2 mg, 0.03 mmol) with hydrogen gas in anhydrous THF (23 ml) and followed by the usual work up. Impurities from crude **90** were partially extracted into hexane and this step was repeated a few times until pure **90** was obtained in 79% yield (860.8 mg). TLC: R_f=0.48 (hexane : ethyl acetate, 4 :1). $[\alpha]_D^{20}$ = +16.2 (c 0.5, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.25 (br s, 36 H), 1.45 (m, 4 H), 2.30 (dd, J=16.0, 6.0 Hz, 1 H), 2.51 (dd, J=16.0, 6.0 Hz, 1 H), 3.19 (m, 1 H), 3.32 (m, 1 H), 3.53 – 3.65 (m, 3 H), 3.75 (dd, J=11.0, 2.0 Hz, 1 H), 3.94 (m, 1 H), 4.17 (m, 1 H), 4.42 (d, J=12.0 Hz, 1 H), 4.45 (m, 1 H, H-4), 4.52 (d, J=12.0 Hz, 1 H), 4.57 (d, J=12.0 Hz, 1 H), 4.72 (d, J=12.0 Hz, 1 H), 4.86 – 4.95 (m, 4 H), 5.28 (dd, J=3.5, 3.5 Hz, 1 H, H-1), 5.33 (d, J=10.0 Hz, 1 H, NH), 5.40 (dd, J=10.5, 9.5 Hz, 1 H, H-3), 7.30 (m, 15 H, Ar-H).

Example 81 Preparation of Compound **91**

In a similar method as described for **43**, compound **90** (900 mg, 0.82 mmol) was treated with trichloroacetonitrile (1 ml) and DBU (4 drops) at room temperature for 2 h. The solvent was

removed and the residue purified by flash silica gel chromatography (ethyl acetate : hexane, 1 : 4 and 1 : 3.5, with 0.5% triethyl amine) to give **91** (626 mg, 61%). TLC: R_f=0.30 (ethyl acetate : hexane, 1 : 3). $[\alpha]_D^{20}$= +42.7 (c 2.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.25 (br s, 36 H), 1.40 (m, 4 H), 2.37 (dd, J=15.5, 6.0 Hz, 1 H), 2.53 (dd, J=15.5, 6.0 Hz, 1 H), 3.21 (m, 1 H), 3.34 (m, 1 H), 3.57 (m, 1 H), 3.73 (d, J=2.5 Hz, 2 H), 4.04 (m, 1 H), 4.15 (ddd, J=9.5, 8.5, 3.5 Hz, 1 H, H-2), 4.45 (d, J=12.0, Hz, 1 H), 4.50 (d, J=12.0 Hz, 1 H). 4.53 (d, J=12.0 Hz, 1 H), 4.80 (m, 1 H), 4.83 (d, J=12.0 Hz, 1 H), 4.95 (m, 4 H), 5.30 (d, J=8.5 Hz, 1 H, NH), 5.43 (dd, J=10.5, 9.5 Hz, 1 H, H-3), 6.45 (d, J=3.5 Hz, 1 H, H-1), 7.30 (m, 15 H, Ar-H), 8.75 (s, 1 H, NH).

Example 82 Preparation of Compound **92**

In a similar method as described for **44,** the mixture of imidate **91** (221.1 mg, 0.17 mmol), compound 79 (109.7 mg, 0.15 mmol) and molecular sieves (4 Å, 500 mg) in anhydrous CH_2Cl_2 (4 ml) was treated with diluted BF_3 diethyl etherate solution (75 μl). This diluted BF_3 etherate solution was prepared by diluting 50 μl BF_3 etherate solution with 300 μl of anhydrous CH_2Cl_2. Followed by usual work up and silica gel chromatography (ethyl acetate : CH_2Cl_2 : hexane, 1 : 1 : 2) afforded pure compound **92** (183.1 mg, 68%). TLC: R_f=0.35 (ethyl acetate : hexane, 1 : 2). $[\alpha]_D^{20}$= +21.2 (c 0.5, chloroform). ^{1}H NMR (500 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 9 H, 3 CH_3), 1.26 (m, 54 H), 1.42 – 1.60 (m, 6 H), 2.34 (dd, J=16.0, 6.0 Hz, 1 H), 2.44 (dd, J=16.0, 5.0 Hz, 1 H), 2.47 (dd, J=15.0, 5.0 Hz, 1 H), 2.63 (dd, J=15.0, 7.5 Hz, 1 H), 3.11 (d, J=3.5 Hz, 1 H, OH), 3.21 (m, 1 H), 3.35 (m, 1 H), 3.54 (m, 1 H), 3.65 (m, 3 H), 3.82 (m, 4 H), 3.92 (ddd, J=10.0, 10.0, 3,5 Hz, 1 H, H-2), 4.07 (br d, J=9.5 Hz, 1 H), 4.45 (d, J=11.5 Hz, 1 H), 4.47 – 4.61 (m, 7 H), 4.65 (d, J=12.0 Hz, 1 H), 4.65 (m, 1 H), 4.70 (d, J=11.5 Hz, 1 H), 4.80 (d, J=8.0 Hz, 1 H, H-1'), 4.90 (m, 5 H), 5.16 (dd, J=9.5 H, 9.5 Hz, 1 H), 5.26 (d, J=10.0 Hz, 1 H, NH), 5.37 (m, 2 H). 7.30 (m, 25 H, Ar-H).

Example 83 Preparation of Compound **93**

In a similar method as described for **85,** compound **92** (173.9 mg) was converted into its free amino compound with Zn powder (475 mg) and acetic acid (4 ml). The mixture of the amine compound was coupled with lipid acid **20** (251 mg, 0.58 mmol) using DCC (205.83 mg, ≈ 1.0 mmol). After usual work up and silica gel chromatography (acetone : hexane : chloroform,)

provided pure **93** (96.3 mg, 45 %). TLC: R_f=0.30 (hexane : dichloromethane : acetone, 6 : 2 : 1). $[\alpha]_D^{20}$= +22.0 (c 1.0, chloroform). ^{1}H NMR (500 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 21 H, 7 CH_3), 1.24 (m, 126 H), 1.30 – 1.50 (m, 14 H), 2.21 – 2.31 (m, 4 H), 2.32 (dd, J=16.0, 6.5 Hz, 1 H), 2.42 (dd, J=15.0, 5.0 Hz, 1 H), 2.50 (dd, J=15.5, 5.5 Hz, 1 H), 2.61 (dd, J=15.5, 7.0 Hz, 1 H), 3.18 (m, 1 H), 3.28 – 3.41 (m, 5 H), 3.49 – 3.56 (m, 4 H), 3.61 – 3.87 (m, 8 H), 4.02 (m, 1 H), 4.28 (ddd, J=10.5, 9.5, 3.5 Hz, 1 H, H-2), 4.43 (m, 4 H), 4.51 (d, J=11.5 Hz, 1 H), 4.53 (d, J=11.5 Hz, 1 H), 4.69 (d, J=11.5 Hz, 1 H), 4.83 (d, J=8.0 Hz, 1 H, H-1'), 4.89 (m, 5 H), 5.19 (dd, J=10.5, 9.5 Hz, 1 H), 5.36 (dd, J=10.5, 9.5 Hz, 1 H), 6.44 (d, J=9.5 Hz, 1 H, NH), 6.57 (d, J=7.5 Hz, 1 H, NH), 7.30 (m, 25 H, Ar-H).

Example 84 Preparation of Compound **94**

In a similar method as described for **33,** compound **93** (60.8 mg, 0.027 mmol) was converted to **94** which was purified by silica gel chromatography (chloroform : methanol : water : acetic acid, 15 : 1 : 0.1 : 0.1) to give pure **94** (26.9 mg, 55 %) TLC: R_f=0.32 (chloroform : methanol : water : acetic acid, 10 : 1 : 0.1 : 0.1). $[\alpha]_D^{20}$= +15.0 (c 1.0, chloroform). ES-MS calcd for $C_{104}H_{201}N_2O_{20}P$: 1829.5; found: 1829.5 (negative mode, M-H, ^{13}C isotopic peak).

Example 85 Preparation of Compound **95**

Compound **27** (500 mg, 1.03 mmol) and benzyl trichloroacetimidate (521 mg, 2.06 mmol) were dissolved in dry dichloromethane : hexane, 1 : 2 (15 ml) and molecular sieves (4 Å, ~1.0 g) was added. The mixture was stirred at room temperature for 15 min and trifluoromethane sulfonic acid (460 mg, 28 μl, 0.31 mmol) was added. The reaction was continued for 2 h and then triethyl amine (0.2 ml) was added. The solid was filtered off through celite and washed with dichloromethane. The filtrate was concentrated in *vacuo* and the residue purified by flash silica gel chromatography to give **95** (300 mg, 56%). TLC: R_f=0.36 (ethyl acetate : hexane, 1 : 4). $[\alpha]_D^{20}$= +93.8 (c 0.8, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.45 (s, 3 H, CH_3), 1.55 (s, 3 H, CH_3), 3.62 (dd, J=10.0, 8.5 Hz, 1 H), 3.70 – 3.88 (m, 5 H), 3.98 (ddd, J=10.0, 10.0, 3.5 Hz, 1 H), 4.47 (d, J=12.0 Hz, 1 H), 4.63 (d, J=12.0 Hz, 1H), 4.68 (d, J=12.0 Hz, 1 H), 4.71 (d, J=12.0 Hz, 1 H), 4.75 (d, J=12.0 Hz, 1 H), 4.86 (d, J=12.0 Hz, 1 H), 4.93 (d, J=3.5 Hz, 1 H, H-1), 5.09 (d, J=10.0 Hz, 1 H, NH), 7.30 (m, 10 H, Ar-H).

Example 86 Preparation of Compound **96**

Compound **95** (280 mg, 0.49 mmol) was treated with acetic acid : water (4 : 1, 20 ml) at 45°C for 2 h. Usual work-up and flash silica gel chromatography (toluene : acetone, 4 : 1) gave **96** (247 mg, 95%). TLC: R_f=0.20 (toluene : acetone, 3 : 1). $[\alpha]_D^{20}$= +90.0 (c 0.5, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ=2.00 (br s, 1 H, OH), 2.60 (br s, 1 H, OH), 3.62 - 3.73 (m, 3 H), 3.81 (br s, 2 H), 3.98 (ddd, J=10.0, 10.0, 4.0 Hz, 1 H, H-2), 4.50 (d, J=11.5 Hz, 1 H), 4.66 (d, J=12.0 Hz, 1 H), 4.70 (d, J=11.5 Hz, 1 H), 4.72 (d, J=12.0 Hz, 1 H), 4.77 (d, J=11.5 Hz, 1 H), 4.82 (d, J=11.5 Hz, 1 H), 4.93 (d, J=4.0 Hz, 1 H, H-1), 5.19 (d, J=10.0 Hz, 1 H, NH), 7.30 (m, 10 H, Ar-H). ES-MS calcd for $C_{23}H_{26}Cl_3NO_7$: 533.1. Found: 533.8 (M+H).

Example 87 Preparation of Compound **97**

Compound **96** (450 mg, 0.84 mmol) was dissolved in acetic acid (30 mg) and treated with zinc (6.0 g) at room temperature for 6 h. The solid was filtered off and washed with acetic acid, and the filtrate was concentrated in *vacuo*. The residue was passed through a short silica gel column (6% to 8% dichloromethane in methanol) to give **97** (300 mg, 95%). TLC: R_f=0.20 (6% methanol in dichloromethane). $[\alpha]_D^{20}$= +133.0 (c 0.2, methanol). ^1H NMR (300 MHz, CD_3OD): δ=3.10 (dd, J=10.0, 3.5 Hz, 1 H, H-2), 3.57 – 3.82 (m, 5 H), 4.60 (d, J=11.5 Hz, 1 H), 4.69 (d, J=11.0 Hz, 1 H), 4.75 (d, J=11.5 Hz, 1 H), 5.06 (d, J=11.0 Hz, 1 H), 5.23 (d, J=3.5 Hz, 1 H, H-1), 7.35 (m, 10 H, Ar-H).

Example 88 Preparation of Compound **98**

In a similar method as described for **55,** compound **97** (100 mg, 0.28 mmol) was treated with **23** (162 mg, 0.25 mmol) and DCC (209 mg, 1.02 mmol) in dichloromethane : dimethyl formamide (5 : 1, 6 ml). The usual work up followed by flash silica gel chromatography (chloroform : acetone, 9 : 1) afforded **98** (93 mg, 38%). TLC: R_f=0.30 (dichloromethane : methanol, 100 : 2). $[\alpha]_D^{20}$= +52.0 (c 0.4, chloroform). ^1H NMR (300 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 6 H, 2 CH_3), 1.25 (br s, 54 H), 1.45 – 1.60 (m, 6 H), 2.00 (m, 1 H, OH), 2.29 (dd, J=15.5, 5.5 Hz, 1 H), 2.40 (m, 3 H), 2.50 (d, J=1.5 Hz, 1 H, OH), 3.37 (t, J=6.5 Hz, 2 H), 3.61 – 3.80 (m, 5 H), 4.30 (ddd, J=9.5, 9.5, 3.5 Hz, 1 H, H-2), 4.48 (d, J=12.0 Hz, 1 H), 4.65 (d, J=11.5 Hz, 1 H), 4.70 (d, J=12.0 Hz, 1 H), 4.73 (d, J=11.5 Hz, 1 H), 4.89 (d, J=3.5 Hz, 1 H, H-1), 5.07 (m, 1 H), 5.96 (d,

J=8,5 Hz, 1 H, NH), 7.30 (m, 10 H, Ar-H). ES-MS calcd for $C_{60}H_{101}NO_9$: 979.7. Found: 980.9 (M+H).

Example 89 Preparation of Compound **99**

In a similar method as described for **44**, the mixture, **91** (188 mg, 0.15 mmol), **98** (93 mg, 0.10 mmol) molecular sieves (4 Å, 1.0 g) and dry dichloromethane (3 ml) was treated with BF_3 etherate solution (0.05 M in dichloromethane, 0.6 ml). The usual work up followed by repeated flash silica gel chromatography (hexane : ethyl acetate, 2.5 : 1 and 2 : 1; toluene : acetone, 8 : 1) to give **99** (109 mg, 56%). TLC: R_f=0.33 (hexane : ethyl acetate, 2 : 1). $[\alpha]_D^{20}$= +24.0 (c 0.3, chloroform). ^{1}H NMR (500 MHz, $CDCl_3$): δ=0.88 (t, J=6.5 Hz, 15 H, 5 CH_3), 1.25 (m, 90 H), 1.40 – 1.58 (m, 10 H), 2.25 –2.45 (m, 6 H), 2.95 (br s, 1 H, OH), 3.21 (m, 1 H), 3.36 (m, 3 H), 3.47 – 3.53 (m, 2 H), 3.57 – 3.67 (m, 4 H), 3.72 (m, 2 H), 3.80 (m, 2 H), 4.03 (br d, J=11.0 Hz, 1 H), 4.25 (ddd, J=10.0, 9.0, 3 5 Hz, 1 H, H-2), 4.44 (d, J=11.5 Hz, 1 H), 4.48 (m, 3 H), 4.59 (m, 2 H), 4.67 (d, J=11.5 Hz, 1 H), 4.69 (d, J=11.5 Hz, 1 H), 4.75 (d, J=11.5 Hz, 1 H), 4.85 – 4.92 (m, 6 H), 5.04 (m, 1 H), 5.36 (m, 2 H), 5.80 (d, J=9.0 Hz, 1 H, NH), 7.30 (m, 25 H, Ar-H).

Example 90 Preparation of Compound **100**

In a similar method as described for **45**, compound **99** (95 mg, 0.046 mmol) was converted into its free amino compound **100** using zinc powder (2.0 g) and acetic acid (20 ml). The crude compound was freeze dried from dioxane to give **100** (85 mg, 97%). TLC: R_f=0.11 (2% methanol in dichloromethane).

Example 91 Preparation of Compound **101**

In a similar method as described for **45**, the free amino compound, **100** (42 mg, 0.02 mmol) was coupled with lipid acid, **20** (36,8 mg, 0.09 mmol) using DCC (28 mg, 0.13 mmol) in dry dichloromethane (3 ml). The usual work up followed by flash silica gel chromatography (hexane : chloroform : acetone, 6 : 3 : 1.5) afforded **101** (38 mg, 75%). TLC: R_f=0.50 (hexane : chloroform : acetone, 6 : 3 : 2). $[\alpha]_D^{20}$= +21.2 (c 0.4, chloroform). ^{1}H NMR (600 MHz, $CDCl_3$): δ=0.86 (m, 21 H, 7 CH_3), 1.23 – 1.60 (m, 140 H), 2.20 – 2.39 (m, 8 H), 2.47 (dd, J=15.5, 5.5 Hz, 1 H), 3.17 (m, 1 H), 3.31 – 3.39 (m, 5 H), 3.50 – 3.67 (m, 7 H), 3.68 – 3.78 (m, 4 H), 3.84 (dd, J=11.0, 3.5 Hz, 1 H), 3.96 (dd, J=11.0, 3.0 Hz, 1 H), 4.21 (ddd, J=10.5, 9.0, 3.0 Hz, 1 H, H-2),

4.40 (d, J=12.0 Hz, 1 H), 4.43 (d, J=12.0 Hz, 1 H), 4.44 (m, 1 H), 4.47 (d, J=12.0 Hz, 1 H), 4.64 (d, J=12.0 Hz, 1 H), 4.65 (d, J=12.0 Hz, 1 H), 4.87 (m, 7 H), 5.03 (m, 1 H), 5.34 (dd, J=10.5 H, 9.0 Hz, 1 H), 5.75 (d, J=9.0 Hz, 1 H, NH), 6.57 (d, J=9.0 Hz, 1 H, NH), 7.30 (m, 25 H, Ar-H).

Example 92 Preparation of Compound **102**

In a similar method as described for **33,** under hydrogen atmosphere compound **101** (20 mg, 8.78 μmol) was converted to **102** by palladium on charcoal (5%, 50 mg) in THF-acetic acid (10 : 1, 50 ml) for 24 h. Usual work-up and flash silica gel chromatography purification (chloroform : methanol : water : acetic acid, 9 : 1 : 0.1 : 0.1 and 8 : 1 : 0.1 : 0.1) gave **102** (12 mg, 75%). TLC: R_f=0.38 (chloroform : methanol : water : ammonium hydroxide, 7 : 3 : 0.4 : 0.2). $[\alpha]_D^{20}$= +2.0 (c 0.2, chloroform : methanol, 4 : 1). ES-MS calcd for $C_{104}H_{201}N_2O_{20}P$: 1829.4; found: 1829.5 (negative mode, M-H, ^{13}C isotopic peak).

Example 93 Preparation of Compound **103**

In a similar method as described for **101,** compound **100** (42 mg, 0.02 mmol) and **17** (30 mg, 0.09 mmol) were treated with DCC (28 mg, 0.134 mmol) in dry dichloromethane (3 ml) to give **103** (37 mg, 76%) which was purified through repeated flash silica gel chromatography (hexane : ethyl acetone, 2 : 1; hexane : acetone, 3 : 1). TLC: R_f=0.39 (hexane : chloroform : acetone, 6 : 3 : 2). $[\alpha]_D^{20}$= +21.2 (c 0.4, chloroform). ^{1}H NMR (600 MHz, $CDCl_3$): δ=0.86 (m, 18 H, 6 CH_3), 1.23 – 1.60 (m, 120 H), 2.23 (dd, J=15.0, 6.5 Hz, 1 H), 2.25 – 2.39 (m, 7 H), 2.44 (dd, J=15.5, 5.5 Hz, 1 H), 3.17 (m, 1 H), 3.34 (m, 3 H), 3.50 – 3.76 (m, 12 H), 3.89 (dd, J=11.0, 3.0 Hz, 1 H), 4.20 (ddd, J=10.0, 9.0, 3.0 Hz, 1 H, H-2), 4.37 (d, J=11.5 Hz, 1 H), 4.39 – 4.43 (m, 3 H), 4.46 (d, J=11.5 Hz, 1 H), 4.51 (d, J=8.0 Hz, 1 H), 4.53 (d, J=11.5 Hz, 1 H), 4.61 (d, J=11.5 Hz, 1 H), 4.65 (d, J=12.0 Hz, 1 H), 4.82 – 4.90 (m, 6 H), 5.03 (m, 1 H), 5.22 (dd, J=10.0, 9.0 Hz, 1 H), 5.71 (d, J=9.0 Hz, 1 H, NH), 6.38 (d, J=8.0 Hz, 1 H, NH), 7.30 (m, 30 H, Ar-H).

Example 94 Preparation of Compound **104**

In a similar way as described for **102**, compound **103** (16 mg, 7.27 μmol) was treated with palladium on charcoal (50 mg) under hydrogen atmosphere in THF : acetic acid (10 : 1, 50 ml) to give **104** (10.5 mg, 87%) which was purified by flash silica gel chromatography (chloroform : methanol: water : acetic acid, 9 : 1 : 0.1 : 0.1, 8 : 1 : 0.1 : 0.1 and 6 : 1 : 0.1 : 0.1). TLC: R_f=0.20

(chloroform : methanol : water : ammonium hydroxide, 7 : 3 : 0.4 : 0.2). $[\alpha]_D^{20}$= -3.5 (c 0.2, chloroform : methanol, 4 : 1). ES-MS calcd for $C_{92}H_{177}N_2O_{20}P$: 1661.3; found: 1661.3 (negative mode, M-H, ^{13}C isotopic peak).

Example 95 Preparation of Liposomal Formulations

Synthetic Lipid-A analogs **33, 48, 54, 58, 70, 77, 86, 94, 102, 104** and the commercial Lipid-A product **Natural Lipid-A**, were incorporated into liposomal formulations. The **Natural Lipid-A** was purchased from AVANTI and it contained a mixture of Lipid-A analogs extracted from *Salmonella* bacterial cell wall.

Typically, the liposomal formulation was composed of 400 μg of MUC1-based lipopeptide BP1-148, H_2N-STAPPAHGVTSAPDTRPAPGSTAPPK(Pal)G-OH, 200 μg of Lipid-A analog, 6.94 mg of cholesterol, 1.46 mg of dimyristoyl phosphatidylglycerol (DMPC) and 11.62 mg of dipalmitoyl phosphatidylcholine (DPPC) per 1 ml of saline (0.9% NaCl solution).

The liposomal constructs were formulated by first dissolving the phospholipids, cholesterol and Lipid A analog in *tert*-butanol at about 53°C. Lipopeptide and water (5%, v/v) were then added to the *tert*-butanol solution. The resulting clear 95% *tert*-butanol solution was injected into about 4 volumes of rapidly stirred water at about 50°C, using a glass syringe with an 18-gauge needle. The small unilamellar vesicles (SUV) formed in this process were cooled, sterilized by filtration through a 0.22 μm membrane filter, filled into vials and lyophilized. The dry powder was re-hydrated with sterile saline before injection, resulting in the formation of multilamellar large vesicles (MLV). The liposomes formed are used to immunize mice.

Example 96 Mice immunized with Liposomal Vaccines

Groups of C57-Black mice were immunized subcutaneously with the liposomal vaccine containing 40 μg of MUC1-based lipopeptide BP1-148 (FIG. 34), which has the peptide sequence of H_2N-STAPPAHGVTSAPDTRPAPGSTAPPK(Pal)G-OH, and 20 μg of Lipid-A analog per dose. Nine days after vaccine injection mice were sacrificed and lymphocytes were taken from the draining lymph nodes (local response) or from the spleens (systemic response) to determine the immune response in each group. The lymphocytes taken from immunized mice were incubated in *in vitro* cultures in the presence of MUC1-based boosting antigen BP1-151, which has the peptide sequence H_2N-STAPPAHGVTSAPDTRPAPGSTAPPK-OH.

Example 97 Measurement of T-cell Proliferation

T-cell proliferation was evaluated using a standard ^3H thymidine incorporation assay. Briefly, nylon wool passed inguinal lymph node lymphocytes from each mouse were added to a culture containing 10^6 native mitomycin C treated syngeneic splenocytes, which serves as antigen presenting cells (APCs). To each well 20 μg of MUC1-based boosting peptide BP1-151, H_2N-STAPPAHGVTSAPDTRPAPGSTAPPK-OH (single letter amino acid code, Table 8), was added for positive control; and cultures containing no antigen or peptide BP1-72, which has the peptide sequence H_2N-EAIQPGCIGGPKGLPGLPGP-OH, were used as negative control. The culture was incubated for 72 h in a total volume of 250 μl / well, followed by adding 1 μCi of ^3H-thymidine in a volume of 50 μl. The plates were incubated for an additional 18–20 h. Cells were harvested and [^3H]dTh incorporation was measured by liquid scintillation counter. T-cell proliferation results corresponding to various liposomal vaccines adjuvanted with different Lipid-A analogs are summarized in Tables 1 - Table 3 and FIG. 31– FIG. 33.

List of Tables

Table 1 T-cell proliferation and Interferon-gamma production of Lipid-A analogs **33**, **48** and **58**

Lipid-A Analogs	T-cell-Proliferation (CPM)	Interferon-gamma (IFN- γ, pg/ml)
33	822	0
48	14112	2430
58	686	0
Natural Lipid-A	14172	2938
saline	3274	0

Table 2 T-cell proliferation and Interferon-gamma production of Lipid-A analogs **48**, **54**, **70**, **77** and **86**

Lipid-A analogs	T-cell-Proliferation (CPM)	Interferon-gamma (IFN- γ, pg/ml)
48	28149	18261
54	32976	36166
70	25034	17321
77	2565	0
86	52382	36461
Natural Lipid-A	37851	31741
Saline	183	0

Examples 98 Measurement of Interferon-gamma (IFN-γ) Production

Interferon-gamma (IFN-γ) levels were determined in the cell culture supernatants using enzyme-linked immunoabsorbent assay (ELISA). 96-Well plates were coated with 50 μl of catcher Mabs in 50 μl of R4.6AZ at 37°C for 30 min. The plates were then washed and incubated with test samples for 45 min. After two washes the second biotinylated antibody, XMG1.2 was added. After washing, peroxidase-conjugated streptavidin was added and incubated again for 30 min.

Finally, 100 µl of horseradish peroxidase (HRPO) substrate solution was added. The optical density was measured with a Thermomax ELISA reader at 405 nm wavelength in kinetic mode for 10 min. The interferon-gamma (IFN-γ) levels corresponding to various Lipid-A analogs are shown in Table 1 - Table 3 and FIG. 31 – FIG. 33.

Table 3 T-cell proliferation and Interferon-gamma production of Lipid-A analogs **86**, **94**, **102** and **104**

Lipid-A analogs	T-cell-Proliferation (CPM)	Interferon-gamma (IFN- γ, pg/ml)
86	15486	17697
94	10882	2150
102	13389	10587
104	12635	14669
Natural lipid-A	14539	31166
Saline	5078	362

Example 99 Lethal Toxicity of Synthetic and Natural Lipid-A Analogs

Synthetic Lipid-A analog **86** and the **Natural Lipid-A** (see **Example 95** for more detail) were tested for their lethal toxicity. Test methods and results are summarized below (Table 4).

a) Preparation of Lipid-A analog solution: 1 mg of Lipid-A analog was dissolved in 1 ml of 20% DMSO and diluted with saline to obtain 50 µg, 10 µg and 2 µg doses in 0.5 ml.

b) Preparation of Actinomycin D: 5 mg of Actinomycin D was dissolved in 1 ml of ethanol and diluted with saline to obtain a dose at 550 µg/kg in 0.5 ml.

c) Procedure: Various doses of Lipid-A analogs or solvent as control were injected intraperitoneally (i.p.) into C57-Black mice. Twenty minutes later all groups of mice were injected with 500 µl of Actinomycin D. Mice were then observed for mortality or any other symptoms of toxicity.

During the first 24 hour observation period all three mice injected with the high 50 µg dose of **Natural Lipid-A** as well as two mice injected with 10 µg dose of **Natural Lipid-A** were found

dead (Table 4). The groups of mice injected with all three doses of synthetic Lipid-A analog **86**, and mice injected with low 2 μg dose of **Natural Lipid-A** survived the study. No further mouse death was recorded after 24 hours and the experiment was terminated 4 days later. The results of this study showed that the synthetic Lipid-A analog **86** is much less toxic than the **Natural Lipid-A** product tested in this experiment.

Table 4 Comparison of lethal toxicity of synthetic and natural Lipid-A analogs

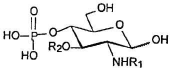
Group	Lipid-A Analog	Dose (μg / μl)	Actinomycin D	No.Survived / No.Tested (+24 h)
1	**86**	50 / 500	+	3 / 3
2	**86**	10 / 500	+	3 / 3
3	**86**	2 / 500	+	3 / 3
4	**Natural**	50 / 500	+	0 / 3
5	**Natural**	10 / 500	+	1 / 3
6	**Natural**	2 / 500	+	3 / 3
7	Solvent	0 / 500	+	3 / 3

The above examples described the detailed procedures for the synthesis of Lipid-A analogs and their intermediates, and the procedures to prepare liposomal vaccines, adjuvanted with synthetic Lipid-A analogs, to induce specific immune response in mice. The common abbreviations used in the text of this invention are listed in Table 5. The structures of Lipid-A analogs prepared and tested for their adjuvanticity are listed in Table 6. A complete list of IUPAC names of all compounds prepared in this invention is given in Table 7. For peptide sequence, single letter codes of amino acids are used in the present invention. For reference, please see the list of standard single letter and three letter codes of naturally occurring amino acids in Table 8.

Table 5 Common abbreviations used in the text

All	allyl
APC	antigen presenting cell
BF_3OEt_2	trifluoroboran diethyl etherate
Bn	benzyl
^{t}Bu	*tert*-butyl
m-CPBA	m-chloroperbenzoic acid
CPM	counts per miniute
DBU	1,8-diazabicyclo[5,4,0]undec-7-ene
DCC	dicyclohexylcarbodiimide
(-)-DIPCl	(-)-B-Chlorodiisopinocamphenylborane
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMPC	dimyristoyl phosphatidyl glycerol
DPPC	dipalmitoyl phosphatidyl choline
DMSO	dimethyl sulfoxide
EDCI	1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride
ES-MS	electron spray mass spectrometry
Et	ethyl
Fmoc	9-fluorenylmethoxylcarbonyl
IFN-γ	interferon-gamma
LPS	lipopolysaccharide
Me	methyl
MLV	multilamellar large vesicles
NBS	N-bromosuccinimide
NMM	N-methyl morpholine
NMR	nuclear magnetic resonance
Pal	palmitoyl
Ph	phenyl
Phth	phthalimido
iPr	isopropyl
Py	pyridine
SUV	small unilamellar vesicles
Tf	trifluoromethylsulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
Troc	trichloroethoxylcarbonyl
Trt	triphenylmethyl
p-TsOH	p-toluenesulfonic acid

Table 6 List of structures of Lipid-A analogs prepared in this invention



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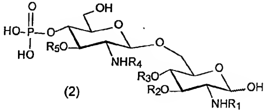
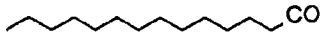
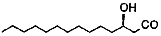


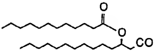
(2)

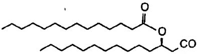
(5) H

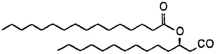
(6) $CH_3CH_2CH_2$

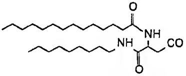
(7) 

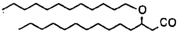
(8) 

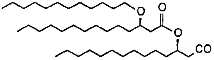
(9) 

(10) 

(11) 

(12) 

(13) 

(14) 

Compounds	Structure	R_1	R_2	R_3	R_4	R_5
33	(1)	(12)	(7)	N/A	N/A	N/A
48	(2)	(11)	(5)	(5)	(9)	(10)
54	(2)	(10)	(5)	(5)	(10)	(10)
58	(2)	(10)	(5)	(5)	(12)	(10)
70	(2)	(10)	(6)	(5)	(10)	(10)
77	(2)	(10)	(8)	(5)	(12)	(10)
86	(2)	(10)	(8)	(5)	(10)	(10)
94	(2)	(13)	(8)	(5)	(13)	(13)
102	(2)	(14)	(5)	(5)	(13)	(13)
104	(2)	(14)	(5)	(5)	(8)	(13)
Natural Lipid-A	(2)	Unidentified mixture	Unidentified mixture	Unidentified mixture	Unidentified mixture	Unidentified mixture

Table 7 List of IUPAC names of compounds prepared in this invention

Compound 1	N-9-Fluorenylmethoxycarbonyl-L-aspartic acid *tert*-butyl ester
Compound 2	(3S)-3-(9-Fluorenylmethoxycarbonyamino)-4-nonylamino-4-oxo-butyric acid *tert*-butyl ester
Compound 3	(3S)-3-Amino-4-nonylamino-4-oxo-butyric acid *tert*-butyl ester
Compound 4	(3S)-3-Tetradecanamido-4-nonylamino-4-oxo-butyric acid *tert*-butyl ester
Compound 5	(3S)-3-Tetradecanamido-4-nonylamino-4-oxo-butyric acid
Compound 6	3-Hydroxytetradecanoic acid ethyl ester
Compound 7	3-Hydroxytetradecanoic acid, or 3-hydroxy-myristic acid
Compound 8	(3R)-3-Hydroxytetradecanoic acid, (3R)-3-hydroxy-myristic acid
Compound 9	(3R)-3-Hydroxytetradecanoic acid phenacyl ester
Compound 10	(3R)-3-Dodecanoyloxytetradecanoic acid phenacyl ester
Compound 11	(3R)-3-Tetradecanoyloxytetradecanoic acid phenacyl ester
Compound 12	(3R)-3-Hexadecanoyloxytetradecanoic acid phenacyl ester
Compound 13	(3R)-3-Dodecanoyloxytetradecanoic acid
Compound 14	(3R)-3-Tetradecanoyloxytetradecanoic acid
Compound 15	(3R)-3-Hexadecanoyloxytetradecanoic acid
Compound 16	(3R)-3-Benzyloxytetradecanoic acid phenacyl ester
Compound 17	(3R)-3-Benzyloxytetradecanoic acid
Compound 18	Dodecyl trifluromethanesulphonate
Compound 19	(3R)-3-Dodecyloxytetradecanoic acid phenacyl ester
Compound 20	(3R)-3-Dodecyloxytetradecanoic acid
Compound 21	(4R)-4-Hydroxypentadecene-1

Compound **22**	(4R)-4-[(3R)-3-Dodecyloxytetradecanoyloxy]-pentadecene-1
Compound **23**	(3R)-3-[(3R)-3-Dodecyloxytetradecanoyloxy]-tetradecanoic acid
Compound **24**	Benzyl 2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside
Compound **25**	Allyl 2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside
Compound **26**	Benzyl 2-deoxy-4,6-di-O-benzylidene-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside
Compound **27**	Benzyl 2-deoxy-4,6-di-O-isopropylidene-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside
Compound **28**	Allyl 2-deoxy-4,6-di-O-benzylidene-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside
Compound **29**	Benzyl 2-deoxy-4,6-di-O-benzylidene-2-(2,2,2-trichloroethoxycarbonylamino)-3-O-tetradecanoyl-α-D-glucopyranoside
Compound **30**	Benzyl 2-deoxy-4,6-di-O-benzylidene-2-[(3S)-3-tetradecanamido-4-nonylamino-4-oxo-butanamido]-3-O-tetradecanoyl-α-D-glucopyranoside
Compound **31**	Benzyl 6-O-benzyl-2-deoxy-2-[(3S)-3-tetradecanamido-4-nonylamino-4-oxo-butanamido]-3-O-tetradecanoyl-α-D-glucopyranoside
Compound **32**	Benzyl 6-O-benzyl-2-deoxy-4-O-(di-O-benzyl-phosphono)-2-[(3S)-3-tetradecanamido-4-nonylamino-4-oxo-butanamido]-3-O-tetradecanoyl-α-D-glucopyranoside
Compound **33**	2-Deoxy-4-O-phosphono-2-[(3S)-3-tetradecanamido-4-nonylamino-4-oxo-butanamido]-3-O-tetradecanoyl-α/β-D-glucopyranose
Compound **34**	Benzyl 2-deoxy-2-phthalimido-β-D-glucopyranoside
Compound **35**	Benzyl 2-deoxy-2-phthalimido-6-O-triphenylmethyl-β-D-glucopyranoside
Compound **36**	Benzyl 2-deoxy-3,4-di-O-benzyl-2-phthalimido-6-O-triphenylmethyl-β-D-glucopyranoside
Compound **37**	Benzyl 2-deoxy-3,4-di-O-benzyl-2-phthalimido-β-D-glucopyranoside
Compound **38**	Benzyl 2-amino-2-deoxyl-3,4-di-O-benzyl-β-D-glucopyranoside

Compound 39	Benzyl 2-deoxy-3,4-di-O-benzyl-2-[(3R)-3-hexadecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound 40	Benzyl 2-deoxy-3,4-di-O-benzyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound 41	Allyl 2-deoxy-4,6-di-O-benzylidene-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside
Compound 42	2-Deoxy-4,6-di-O-benzylidene-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-α/β-D-glucopyranose
Compound 43	2-Deoxy-4,6-di-O-benzylidene-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl trichloroacetimidate
Compound 44	Benzyl 2-deoxy-6-O-{2-deoxy-4,6-di-O-benzylidene-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-3,4-di-O-benzyl-2-[(3R)-3-hexadecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound 45	Benzyl 2-deoxy-6-O-{2-deoxy-4,6-di-O-benzylidene-2-[(3R)-3-dodecanoyloxytetradecanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-3,4-di-O-benzyl-2-[(3R)-3-hexadecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound 46	Benzyl 2-deoxy-6-O-{6-O-benzyl-2-deoxy-2-[(3R)-3-dodecanoyloxytetradecanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-3,4-di-O-benzyl-2-[(3R)-3-hexadecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound 47	Benzyl 2-deoxy-6-O-{6-O-benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-2-[(3R)-3-dodecanoyloxytetradecanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-3,4-di-O-benzyl-2-[(3R)-3-hexadecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound 48	2-Deoxy-6-O-{2-deoxy-2-[(3R)-3-dodecanoyloxytetradecanamido]-4-O-phosphono-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-2-[(3R)-3-hexadecanoyloxytetradecanamido]-α/β-D-glucopyranose

Compound **49**	Benzyl 2-deoxy-6-O-{2-deoxy-4,6-di-O-benzylidene-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-3,4-di-O-benzyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound **50**	Benzyl 2-deoxy-6-O-{2-amino-2-deoxy-4,6-di-O-benzylidene-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-3,4-di-O-benzyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound **51**	Benzyl 2-deoxy-6-O-{2-deoxy-4,6-di-O-benzylidene-2-[(3R)-3-tetradecanoyloxytetradecanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-3,4-di-O-benzyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound **52**	Benzyl 2-deoxy-6-O-{6-O-benzyl-2-deoxy-2-[(3R)-3-tetradecanoyloxytetradecanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-3,4-di-O-benzyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound **53**	Benzyl 2-deoxy-6-O-{6-O-benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-2-[(3R)-3-tetradecanoyloxytetradecanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-3,4-di-O-benzyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound **54**	2-Deoxy-6-O-{2-deoxy-4-O-phosphono-2-[(3R)-3-tetradecanoyloxytetradecanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-2-[(3R)-3-tetradecanoyloxytetradecanamido]-α/β-D-glucopyranose
Compound **55**	Benzyl 2-deoxy-6-O-{2-deoxy-4,6-di-O-benzylidene-2-[(3S)-3-tetradecanamido-4-nonylamino-4-oxo-butanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-3,4-di-O-benzyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound **56**	Benzyl 2-deoxy-6-O-{6-O-benzyl-2-deoxy-2-[(3S)-3-tetradecanamido-4-nonylamino-4-oxo-butanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-3,4-di-O-benzyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound **57**	Benzyl 2-deoxy-6-O-{6-O-benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-2-[(3S)-3-tetradecanamido-4-nonylamino-4-oxo-butanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-3,4-di-O-benzyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside

Compound **58**	2-Deoxy-6-O-{2-deoxy-2-[(3S)-3-tetradecanamido-4-nonylamino-4-oxo-butanamido]-4-O-phosphono-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-2-[(3R)-3-tetradecanoyloxytetradecanamido]-α/β-D-glucopyranose
Compound **59**	Benzyl 2-deoxy-4,6-di-O-benzylidene-2-phthalimido-β-D-glucopyranoside
Compound **60**	Benzyl 3-O-allyl-2-deoxy-6-O-triphenylmethyl-2-phthalimido-β-D-glucopyranoside
Compound **61**	Benzyl 3-O-allyl-4-O-benzyl-2-deoxy-6-O-triphenylmethyl-2-phthalimido-β-D-glucopyranoside
Compound **62**	Benzyl 3-O-allyl-4-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside
Compound **63**	Benzyl 3-O-allyl-2-amino-4-O-benzyl-2-deoxy-β-D-glucopyranoside
Compound **64**	Benzyl 3-O-allyl-4-O-benzyl-2-deoxy-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound **65**	Benzyl 3-O-allyl-4-O-benzyl-2-deoxy-6-O-{2-deoxy-4,6-di-O-benzylidene-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound **66**	Benzyl 3-O-allyl-6-O-{2-amino-2-deoxy-4,6-di-O-benzylidene-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-4-O-benzyl-2-deoxy-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound **67**	Benzyl 3-O-allyl-4-O-benzyl-2-deoxy-6-O-{2-deoxy-4,6-di-O-benzylidene-2-[(3R)-3-tetradecanoyloxytetradecanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound **68**	Benzyl 3-O-allyl-4-O-benzyl-2-deoxy-6-O-{2-deoxy-6-O-benzyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound **69**	Benzyl 3-O-allyl-4-O-benzyl-2-deoxy-6-O-{2-deoxy-6-O-benzyl-4-O-(di-O-benzyl-phosphono)-2-[(3R)-3-tetradecanoyloxytetradecanamido]-3-O-

[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside

Compound 70	2-Deoxy-6-O-{2-deoxy-2-[(3R)-3-tetradecanoyloxytetradecanamido]-4-O-phosphono-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-3-O-propyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-α/β-D-glucopyranose
Compound 71	Benzyl 4-O-benzyl-2-deoxy-6-O-{2-deoxy-4,6-di-O-benzylidene-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound 72	Benzyl 4-O-benzyl-3-O-[(3R)-3-benzyloxytetradecanoyl]-2-deoxy-6-O-{2-deoxy-4,6-di-O-benzylidene-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound 73	Benzyl 6-O-{2-amino-2-deoxy-4,6-di-O-benzylidene-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-4-O-benzyl-3-O-[(3R)-3-benzyloxytetradecanoyl]-2-deoxy-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound 74	Benzyl 4-O-benzyl-3-O-[(3R)-3-benzyloxytetradecanoyl]-2-deoxy-6-O-{2-deoxy-4,6-di-O-benzylidene-2-[(3S)-3-tetradecanamido-4-nonylamino-4-oxo-butanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound 75	Benzyl 4-O-benzyl-3-O-[(3R)-3-benzyloxytetradecanoyl]- 6-O-{6-O-benzyl-2-deoxy-2-[(3S)-3-tetradecanamido-4-nonylamino-4-oxo-butanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-2-deoxy-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound 76	Benzyl 4-O-benzyl-3-O-[(3R)-3-benzyloxytetradecanoyl]-6-O-{6-O-benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-2-[(3S)-3-tetradecanamido-4-nonylamino-4-oxo-butanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-2-deoxy-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside
Compound 77	2-Deoxy-6-O-{2-deoxy-2-[(3S)-3-tetradecanamido-4-nonylamino-4-oxo-butanamido]-4-O-phosphono-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-3-O-[(3R)-3-

	hydroxytetradecanoyl]-2-[(3R)-3-tetradecanoyloxytetradecanamido]-α/β-D-glucopyranose
Compound **78**	Benzyl 3-O-[(3R)-3-benzyloxytetradecanoyl]-2-deoxy-4,6-di-O-isopropylidene-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside
Compound **79**	Benzyl 3-O-[(3R)-3-benzyloxytetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside
Compound **80**	Allyl 6-O-benzyl-2-deoxy-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside
Compound **81**	Allyl 6-O-benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside
Compound **82**	6-O-Benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-α/β-D-glucopyranose
Compound **83**	6-O-Benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl trichloroacetimidate
Compound **84**	Benzyl 6-O-{6-O-benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-3-O-[(3R)-3-benzyloxytetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside
Compound **85**	Benzyl 6-O-{6-O-benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranosyl}-3-O-[(3R)-3-benzyloxytetradecanoyl]-2-deoxy-2-[(3R)-3-tetradecanoyloxytetradecanamido]-α-D-glucopyranoside
Compound **86**	2-Deoxy-6-O-{2-deoxy-4-O-phosphono-2-[(3R)-3-tetradecanoyloxytetradecanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-3-O-[(3R)-3-hydroxytetradecanoyl]-2-[(3R)-3-tetradecanoyloxytetradecanamido]-α/β-D-glucopyranose
Compound **87**	Allyl 2-deoxy-4,6-di-O-benzylidene-3-O-[(3R)-3-docyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside

Compound **88**	Allyl 6-O-benzyl-2-deoxy-3-O-[(3R)-3-dodecyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside
Compound **89**	Allyl 6-O-benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-3-O-[(3R)-3-dodecyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside
Compound **90**	6-O-Benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-3-O-[(3R)-3-dodecyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-α/β-D-glucopyranose
Compound **91**	6-O-Benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-3-O-[(3R)-3-dodecyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl trichloroacetimidate
Compound **92**	Benzyl 6-O-{6-O-benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-3-O-[(3R)-3-dodecyloxytetradecanoyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-3-O-[(3R)-3-benzyloxytetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside
Compound **93**	Benzyl 6-O-{6-O-benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-3-O-[(3R)-3-dodecyloxytetradecanoyl]-2-[(3R)-3-dodecyloxytetradecanamido]-β-D-glucopyranosyl}-3-O-[(3R)-3-benzyloxytetradecanoyl]-2-deoxy-2-[(3R)-3-dodecyloxytetradecanamido]-α-D-glucopyranoside
Compound **94**	2-Deoxy-6-O-{2-deoxy-4-O-phosphono-2-[(3R)-3-dodecyloxytetradecanamido]-3-O-[(3R)-3-dodecyloxytetradecanoyl]-β-D-glucopyranosyl}-3-O-[(3R)-3-hydroxytetradecanoyl]-2-[(3R)-3-dodecyloxytetradecanamido]-α/β-D-glucopyranose
Compound **95**	Benzyl 3-O-benzyl-2-deoxy-4,6-di-O-isopropylidene-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside
Compound **96**	Benzyl 3-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside
Compound **97**	Benzyl 2-amino-3-O-benzyl-2-deoxy-α-D-glucopyranoside
Compound **98**	Benzyl 3-O-benzyl-2-deoxy-2-{(3R)-3-[(3R)-3-dodecyloxytetradecanoyloxy]-tetradecanamido}-α-D-glucopyranoside
Compound **99**	Benzyl 3-O-benzyl-6-O-{6-O-benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-3-O-[(3R)-3-dodecyloxytetradecanoyl]-2-(2,2,2-

trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-2-deoxy-2-{(3R)-3-[(3R)-3-dodecyloxytetradecanoyloxy]-tetradecanamido}-α-D-glucopyranoside

Compound **100**	Benzyl 6-O-{2-amino-6-O-benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-3-O-[(3R)-3-dodecyloxytetradecanoyl]-β-D-glucopyranosyl}-3-O-benzyl-2-deoxy-2-{(3R)-3-[(3R)-3-dodecyloxytetradecanoyloxy]-tetradecanamido}-α-D-glucopyranoside
Compound **101**	Benzyl 3-O-benzyl-6-O-{6-O-benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-2-[(3R)-3-dodecyloxytetradecanamido]-3-O-[(3R)-3-dodecyloxytetradecanoyl]-β-D-glucopyranosyl}-2-deoxy-2-{(3R)-3-[(3R)-3-dodecyloxytetradecanoyloxy]-tetradecanamido}-α-D-glucopyranoside
Compound **102**	2-Deoxy-6-O-{2-deoxy-4-O-phosphono-2-[(3R)-3-dodecyloxytetradecanamido]-3-O-[(3R)-3-dodecyloxytetradecanoyl]-β-D-glucopyranosyl}-2-{(3R)-3-[(3R)-3-dodecyloxytetradecanoyloxy]-tetradecanamido}-α/β-D-glucopyranose
Compound **103**	Benzyl 3-O-benzyl-6-O-{6-O-benzyl-4-O-(di-O-benzyl-phosphono)-2-deoxy-2-[(3R)-3-benzyloxytetradecanamido]-3-O-[(3R)-3-dodecyloxytetradecanoyl]-β-D-glucopyranosyl}-2-deoxy-2-{(3R)-3-[(3R)-3-dodecyloxytetradecanoyloxy]-tetradecanamido}-α-D-glucopyranoside
Compound **104**	2-Deoxy-6-O-{2-deoxy-4-O-phosphono-2-[(3R)-3-hydroxytetradecanamido]-3-O-[(3R)-3-dodecyloxytetradecanoyl]-β-D-glucopyranosyl}-2-{(3R)-3-[(3R)-3-dodecyloxytetradecanoyloxy]-tetradecanamido}-α/β-D-glucopyranose

Table 8 Single letter and three letter codes for amino acids

Amino acid	Single letter code	Three letter code
L-Alanine	A	Ala
L-Cysteine	C	Cys
L-Asparatate	D	Asp
L-Glutamate	E	Glu
L-Phenylalanine	F	Phe
L-Glycine	G	Gly
L-Histidine	H	His
L-Isoleucine	I	Ile
L-Lysine	K	Lys
L-Leucine	L	Leu
L-Methionine	M	Met
L-Asparagine	N	Asn
L-Proline	P	Pro
L-Glutamine	Q	Gln
L-Arginine	R	Arg
L-Serine	S	Ser
L-Threonine	T	Thr
L-Valine	V	Val
L-Tryptophan	W	Trp
L-Tyrosine	Y	Tyr

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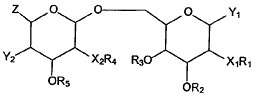
No admission is made that any cited reference constitutes prior art. All references cited anywhere in this specification, including patents, published patent applications, and nonpatent publications, are hereby incorporated by reference in their entirety, as are any references cited by said cited references.

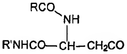
CLAIMS

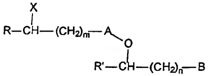
We claim:

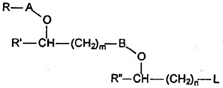
1. A compound of the following formula:



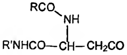
wherein at least one of R_1, R_2, R_3, R_4, R_5 is chosen from the following structures

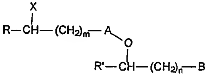


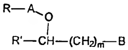


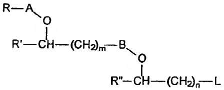


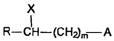
where the remaining R_1, R_2, R_3, R_4, and/or R_5, if any, are selected from the group consisting of hydrogen, -R, -COR and the following structures











where each R, R', R" is independently chosen to be a hydrogen, or a substituted or unsubstituted, branched or linear C_{1-20} saturated or unsaturated aliphatic hydrocarbon;

A, B and L are independently selected from the group consisting of $-CH_2-$, $-C(=O)-$ and $-C(=S)-$ groups;

each X is independently selected from the group consisting of -OH, -SH, $-NH_2$, and -halogen;

m and n are independently selected from the range of integers between 0 and 10 inclusive,

X_1 and X_2 are –O-- or --NH--,

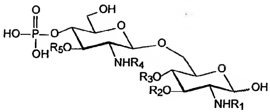
Y_1 and Y_2 are independently selected from the group consisting of -OH, $-OP(O)(OH)_2$, -COOH, $-OSO_3H$, $-CH(COOH)_2$ and $-OP(O)(OH)(OCH_2CH_2NH_2)$,

Z is H, $-CH_2E$, or $-CH_2MG$, where E is -hydrogen, -halogen, -OH, $-NH_2$, $-OSO_3H$, $-SO_3H$, $-P(O)(OH)_2$ or $-OP(O)(OH)_2$; M is –O-, -S-, -OC(=O)-, -SC(=O)-, -OC=(S)-, or -NHC(=O)-; G is a -hydrogen, or a substituted or unsubstituted, branched or linear, saturated or unsaturated C_{1-20} aliphatic hydrocarbon.

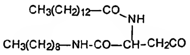
or a physiologically acceptable salt thereof.

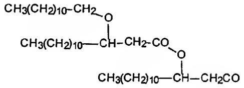
2. A compound of claim 1 where X_1 and X_2 are NH; Y_1 is -OH or $-OP(O)(OH)_2$; Y_2 is -OH or $-OP(O)(OH)_2$; and Z is $-CH_2OH$.

3. A compound of claim 1 where the substitution groups and the stereochemistry of substitutions on the sugar rings are defined by the following formula:

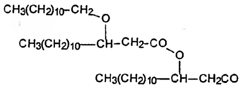


4. A compound of claim 3 where at least one of R_1, R_2, R_3, R_4 and R_5 is chosen from the following groups



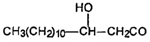


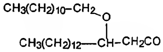
5. A compound of claim 4 where R_1 is the following group



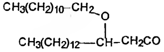
R_2 and R_3 are hydrogen atoms;

R_4 is chosen from the following groups

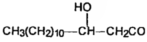




and R_5 is the following group

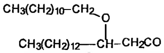


6. A compound of claim 5 where R_4 is the following group



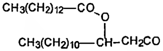
and the absolute configuration of the chiral carbons in group R_1, R_4 and R_5 is (R).

7. A compound of claim 5 where R_4 is the following group

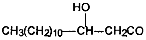


and the absolute configuration of the chiral carbons in group R_1, R_4 and R_5 is (R).

8. A compound of claim 4 where R_1 and R_5 are a group of the following structure

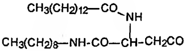


and R_2 is a hydrogen atom or the following group



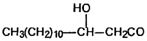
R_3 is a hydrogen atom;

and R_4 is a group of the following structure



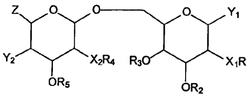
9. A compound of claim 8 where R_2 is a hydrogen atom, and the absolute configuration of the chiral carbons in group R_1 and R_5 is (R), while that in group R_4 is (S).

10. A compound of claim 8 where R_2 is a group of the following structure

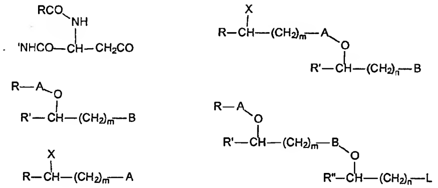


and the absolute configuration of the chiral carbons in group R_1, R_2 and R_5 is (R), while that in group R_4 is (S).

11. A compound of the following formula:



where R_1=R_4 =R_5 and R_1, R_2, R_3, R_4 and R_5 are chosen from -R, -COR and the following structures



where each R, R', R" is independently a hydrogen, substituted or unsubstituted, branched or linear, saturated or unsaturated C_{1-20} aliphatic hydrocarbon; A, B and L are independently CH_2, CO and CS groups; X is -OH, -SH, $-NH_2$ and halogen; m and n are integers between 0 and 10 inclusive.

X_1 and X_2 are independently O and NH.

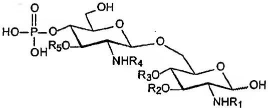
Y_1 and Y_2 are independently group -OH, $-OP(O)(OH)_2$, -COOH, $-OSO_3H$, $-CH(COOH)_2$ and $-OP(O)(OH)(OCH_2CH_2NH_2)$.

Z is H, $-CH_2E$, $-CH_2MG$ where E is hydrogen, halogen, -OH, $-NH_2$, $-OSO_3H$, $-SO_3H$, $-P(O)(OH)_2$ or $-OP(O)(OH)_2$; M is O, S, OC(O), SC(O), OC(S), or NHC(O); G is a hydrogen, substituted or unsubstituted, branched or linear, saturated or unsaturated C_{1-20} aliphatic hydrocarbon.

The said compound can be a free acid or a physiologically acceptable salt.

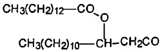
12. A compound of claim 11 where X_1 and X_2 are NH; Y_1 is -OH or -OP(O)(OH)$_2$; Y_2 is -OH or -OP(O)(OH)$_2$; and Z is -CH$_2$OH.

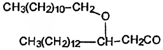
13. A compound of claim 11 where the substitution groups and the stereochemistry of substitutions on the sugar rings are defined by the following formula:



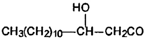
where R_1=R_4=R_5

14. A compound of claim 13 where R_1=R_4=R_5 and are chosen from the following structures



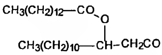


and R_2 is a hydrogen atom, n-propyl group or the following structure



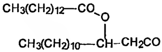
and R_3 is a hydrogen atom.

15. A compound of claim 14 where R_2 is a hydrogen atom, and R_1, R_4, and R_5 are the same group of the following structure



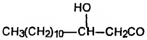
and the absolute configuration of the chiral carbons in group R_1, R_4, and R_5 is (R).

16. A compound of claim 14 where R_2 is an n-propyl group, and R_1, R_4, and R_5 are the same group of the following structure

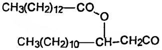


and the absolute configuration of the chiral carbons in group R_1, R_4, and R_5 is (R).

17. A compound of claim 14 where R_2 is the following group

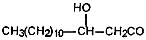


and R_1, R_4, and R_5 are the same group of the following structure

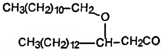


and the absolute configuration of the chiral carbons in group R_1, R_2, R_4, and R_5 is (R).

18. A compound of claim 14 where R_2 is the following group

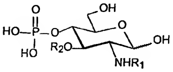


and R_1, R_4, and R_5 are the same group of the following structure

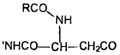


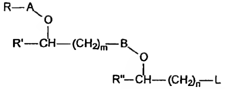
and the absolute configuration of the chiral carbons in group R_1, R_2, R_4, and R_5 is (R).

19. A compound of the following formula:



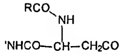
wherein at least one of R_1 and R_2 is chosen from the following groups

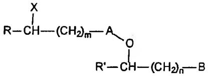


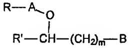


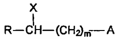
where each R, R', R" is independently a hydrogen, substituted or unsubstituted, branched or linear, saturated or unsaturated C_{1-20} aliphatic hydrocarbon; A, B and L are independently CH_2 and CO groups; m and n are integers between 0 and 10 inclusive.

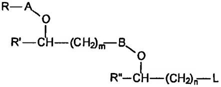
The remaining R_1 or R_2 is chosen from hydrogen, group -R, -COR or the following structures





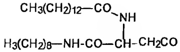


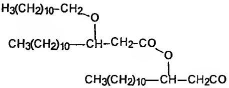




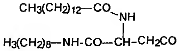
where each R, R', R" is independently a hydrogen, substituted or unsubstituted, branched or linear, saturated or unsaturated C_{1-20} aliphatic hydrocarbon; A, B and L are independently CH_2, CO and CS groups; X is -OH, -SH, $-NH_2$ and halogen; m and n are integers between 0 and 10 inclusive.

20. A compound of claim 19 where at least one of R_1 and R_2 is chosen from the following structures





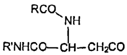
21. A compound of claim 20 where R_1 is a group of the following structure

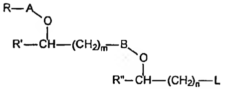


and R_2 is is a group of $CH_3(CH)_{12}CO$

22. A compound of Claim 21 where the absolute configuration of the chiral carbon in group R_1 is (S).

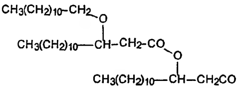
23. A compound that comprises a substitution group of at least one of the following structures



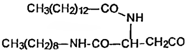


where each R, R', R" is independently selected from the group consisting of hydrogen, and substituted or unsubstituted, branched or linear, saturated or unsaturated $C_{1\text{-}20}$ aliphatic hydrocarbon; A, B and L are independently selected from the group consisting of $-CH_2-$, $-C(=O)-$, and $-C(=S)-$ groups; and m and n are independently selected integers between 0 and 10 inclusive.

24. A compound of claim 23 where the said substitution group has the following structure



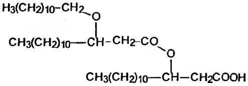
25. A compound of claim 23 where the said substitution group has the following structure



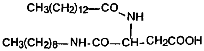
26. A compound of claim 24 where the absolutee configuration of the chiral carbons in the said substitution group is (R).

27. A compound of claim 25 where the absolute configuration of the chiral carbon in the said substitution group is (S).

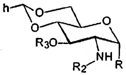
28. A compound of the following structure



29. A compound of the following structure

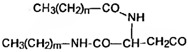


30. A compound of the following formula:



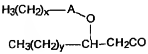
wherein R_1 is a group of benzyloxy, allyloxy, hydroxyl, or $OC(NH)CCl_3$;

R_2 is chosen from the group consisting of hydrogen, $-COOCH_2CCl_3$, or the following structure



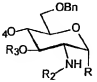
wherein m and n are independently selected integers having values between 0 and 20;

and R_3 is $CH_3(CH_2)_zCO$ or the following structure



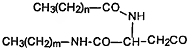
wherein A is $-CH_2-$ or $-C(=O)-$, and x, y and z are independently selected integers having values between 0 and 20.

31. A compound of the following formula:



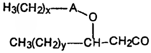
wherein R_1 is a group of benzyloxy, allyloxy, hydroxyl, or $OC(NH)CCl_3$;

R_2 is chosen from a hydrogen atom, $COOCH_2CCl_3$ or



wherein m and n are independent integers having values between 0 and 20;

R_3 is $CH_3(CH_2)_zCO$ or the following structure:

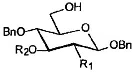


wherein A is $-CH_2-$ or $-C(=O)-$;

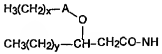
x, y and z are independently selected integers having values between 0 and 20; and

R_4 is hydrogen or $P(O)(OBn)_2$.

32. A compound of the following formula:



wherein R_1 is an amino radical, a phthalamido radical, or the following structure

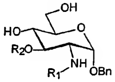


herein A is $-CH_2-$ or $-C(=O)-$;

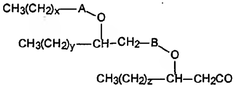
x and y are independently selected integers having values between 0 and 20.

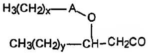
R_2 is allyl or benzyl.

33. A compound of the following formula:



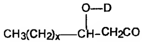
wherein R_1 is hydrogen, $COOCH_2CCl_3$ or one of the following structures





wherein A and B are independently either $-CH_2-$ or $-C(=O)-$; x, y and z are independently selected integers having values between 0 and 20;

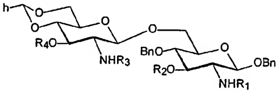
R_2 is benzyl or the following structure

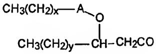


wherein D is a benzyl, $CH_3(CH_2)_z$ or $CH_3(CH_2)_yCO$;

x, y and z are independently selected integers having values between 0 and 20.

34. A compound of the following formula:

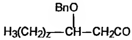




wherein R_1 is a group of the following structure

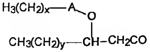
wherein A is $-CH_2-$ or $-C(=O)-$; x and y are independently selected integers having values between 0 and 20;

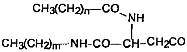
R_2 is hydrogen , allyl, benzyl or the following structure



wherein z is an integer between 0 and 20;

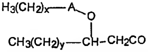
R_3 is hydrogen, $COOCH_2CCl_3$, or one of the following structures





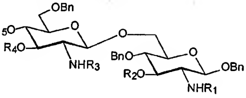
wherein A is $-CH_2-$ or $-C(=O)-$; m, n, x and y are independently selected integers having values between 0 and 20;

R_4 is a group of the following structure

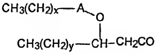


wherein A is $-CH_2-$ or $-C(=O)-$; x and y are independently selected integers having values between 0 and 20.

35. A compound of the following formula:

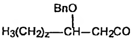


wherein R_1 is a group of the following structure



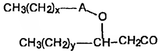
wherein A is $-CH_2-$ or $-C(=O)-$; x and y are independently selected integers having values between 0 and 20;

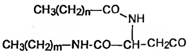
R_2 is hydrogen, allyl, benzyl or the following structure



wherein z is an integer between 0 and 20;

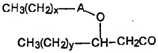
R_3 is hydrogen, $COOCH_2CCl_3$ or one of the following structures





wherein A is $-CH_2-$ or $-C(=O)-$; m, n, x and y are independently selected integers having values between 0 and 20;

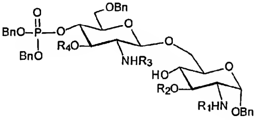
R_4 is a group of the following structure



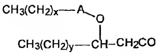
wherein A is $-CH_2-$ or $-C(=O)-$; x and y are independently selected integers having values between 0 and 20;

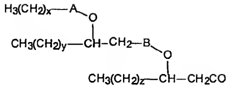
R_5 is hydrogen or $(BnO)_2P(O)$.

36. A compound of the following formula:



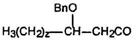
wherein R_1 is chosen from the following structures





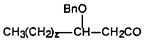
wherein A and B are independently $-CH_2-$ or $-C(=O)-$; x, y and z are independently selected integers having values between 0 and 20;

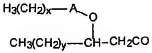
R_2 is a group of benzyl or the following structure



wherein z is an integer between 0 and 20;

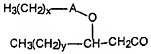
R_3 is hydrogen, $COOCH_2CCl_3$ or the following structure





wherein A is CH_2 or CO; x, y and z are independent integers having values between 0 and 20;

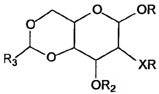
R_4 is a group of the following structure



wherein A is CH_2 or CO; x and y are independent integers having values between 0 and 20.

37. A process to introduce a phosphate group into the 4-O-position of a hexopyranose derivative, where the said process comprises (1) regioselective reductive ring opening of a hexapyranose derivative, to yield a reactive 4-O position and (2) introduction of a phosphate group at said reactive 4-O position.

38. The process of claim 37 where step (1) comprises adding a boron reagent and an acid to a compound with the following structure:



where X is O and NH; R is a substituted or unsubstituted, branched or linear, saturated or unsaturated C_{1-20} aliphatic hydrocarbon, a carbohydrate unit or any protection group; R_1 and R_2 are any aliphatic or aromatic substitution group, or any protection group; R_3 is a phenyl or substituted phenyl group.

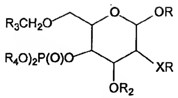
39. The process of claim 38 where said boron reagent is sodium cyanoboron hydride or a dimethyl amine borane complex.

40. The process of claim 38 in which said acid is hydrochloric acid.

41. The process of claim 40 in which said acid is provided in a saturated diethyl ether soluton, trifluoroboron diethyl ether complex

The process of claim 37 in which, in step (2), the product formed in step (1) is treated with reagents $(R_4O)_2PN(iPr)_2$, 1*H*-tetrazole in a dry organic solvent, where R_4 is an allyl, or a substituted or unsubstituted benzyl or phenyl group.

42. The process of claim 37 wherein, after step (2), an oxidizing reagent is added to yield a product with the following structure:



where X, R, R_1, R_2 and R_3 are defined as in the starting material in step (1), R_4 is an allyl, or a substituted or unsubstituted benzyl or phenyl group.

43. The process of claim 42 in which said oxidizing reagent is meta-chloroperbenzoic acid.

44. A non-naturally occurring liposome, whose membrane comprises: (a) a compound according to any of claims 1-36 above; and (b) at least one epitope.

45. The liposome of claim 44 in which at least one epitope is a B-cell epitope.

46. The liposome of claim 44 in which at least one epitope is a T-cell epitope.

47. The liposome of claim 44 in which at least one epitope is a peptide epitope.

48. The liposome of claim 44 in which at least one epitope is a carbohydrate, glycopeptide or glycolipid epitope.

49. A pharmaceutical composition comprising a liposome according to claim 43, said composition comprising a vaccinologically effective amount of said antigen.

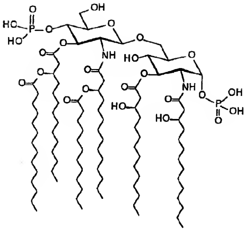
50. The liposome of claim 44 where said antigen is a tumor associated antigen.

51. The liposome of claim 44 where at least one epitope is derived from MUC1 protein.

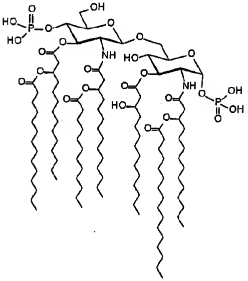
52. The liposome of claim 51 wherein the epitope is provided by a peptide or lipopeptide which has the amino acid sequence of

H_2N-STAPPAHGVTSAPDTRPAPGSTAPPK(Pal)G-OH.

53. Use of the liposome of claim 51 in the manufacture of a composition for the prevention or treatment of a disease preventable or treatable by eliciting an immune response to said antigen.
54. The use of claim 53 in which said lipid A analogue has an adjuvanting effect on the immune response to said antigen.
55. The use of claims 53 or 54 in which said disease is a cancer.

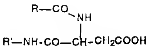


A

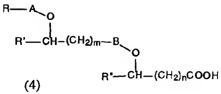


B

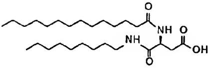
FIG. 1 Examples of natural Lipid-A structures, **A** from *E. coli* and **B** from *Salmonella* strains



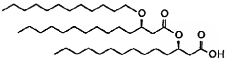
(3)



(4)

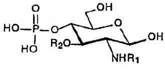


5

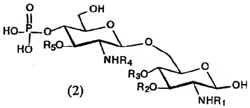


23

FIG. 2 New lipid acids (3) and (4) and two examples thereof

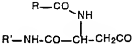


(1)

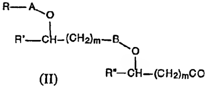


(2)

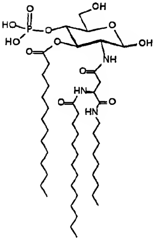
at least one of R_1, R_2, R_3, R_4 and R_5 has the lipid structure (I) or (II)



(I)



(II)



33

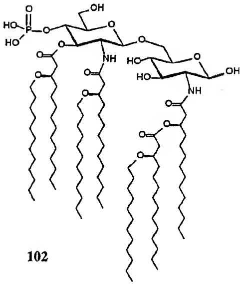


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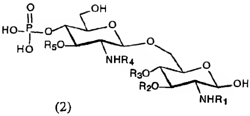
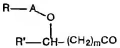
FIG. 3 Monosaccharide and disaccharide Lipid-A analogs containing new lipid structures and two examples thereof

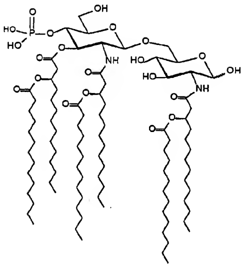


(2)



(III)

R_1, R_4 and R_5 are the same substitution group of structure (III)



54

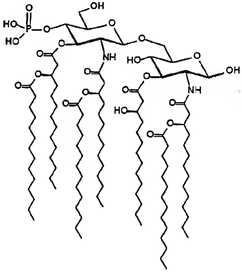


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FIG. 4 Disaccharide Lipid-A analogs with uniform di-lipid chains and two examples thereof

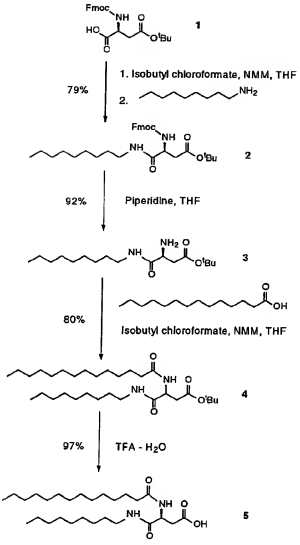


FIG. 5 Synthesis of lipid **5**

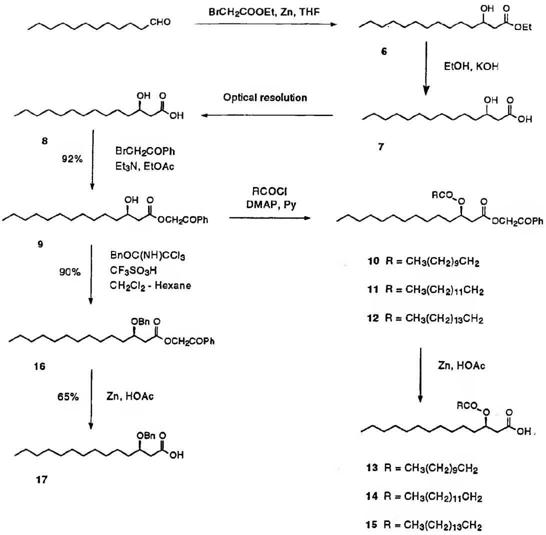


FIG. 6 Synthesis of lipids

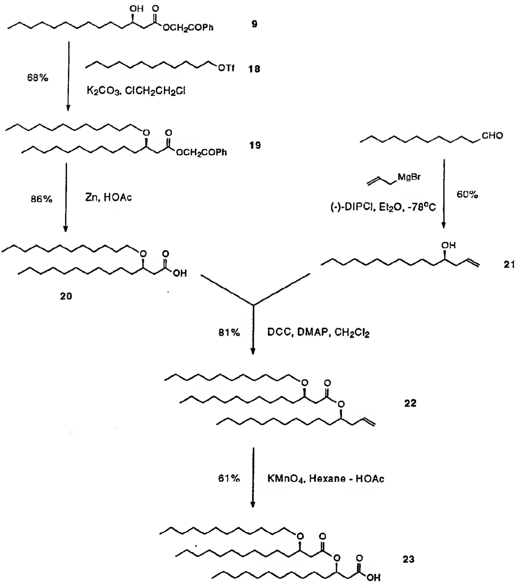


FIG. 7 Synthesis of tri-lipid acid **23**

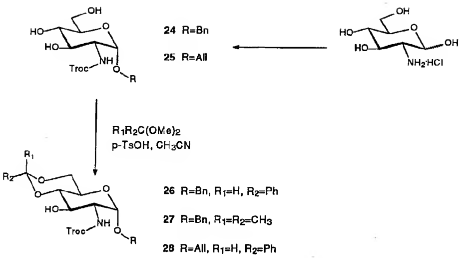


FIG. 8 Synthesis of glucosamine derivatives

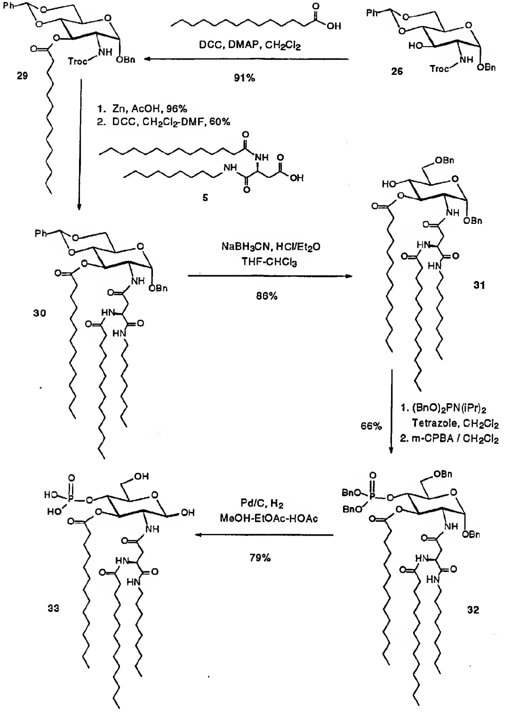


FIG. 9 Synthesis of compound **33**

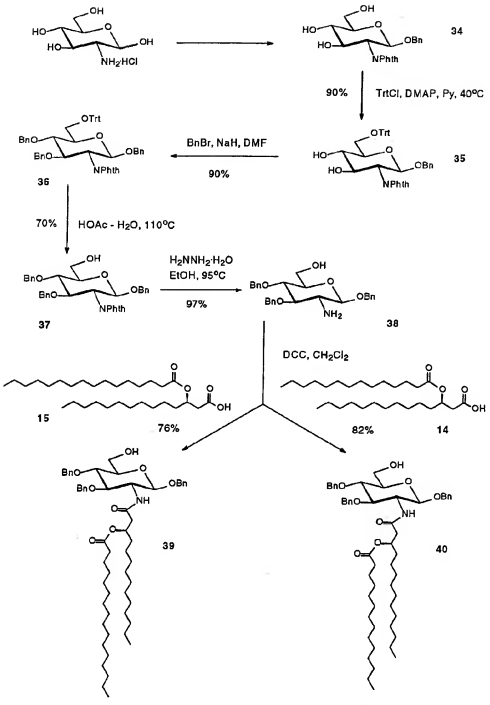


FIG. 10 Synthesis of glycosylation acceptors

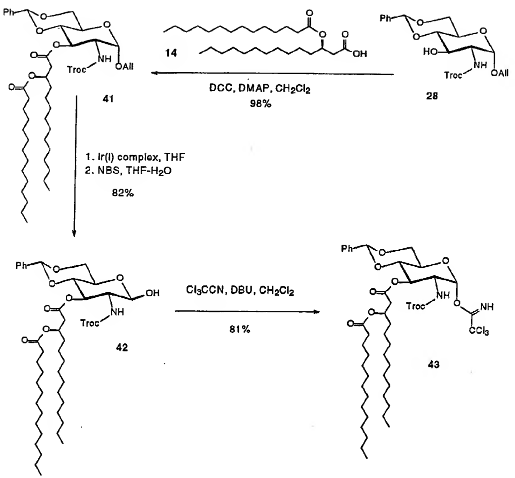


FIG. 11 Synthesis of glycosylation donor **43**

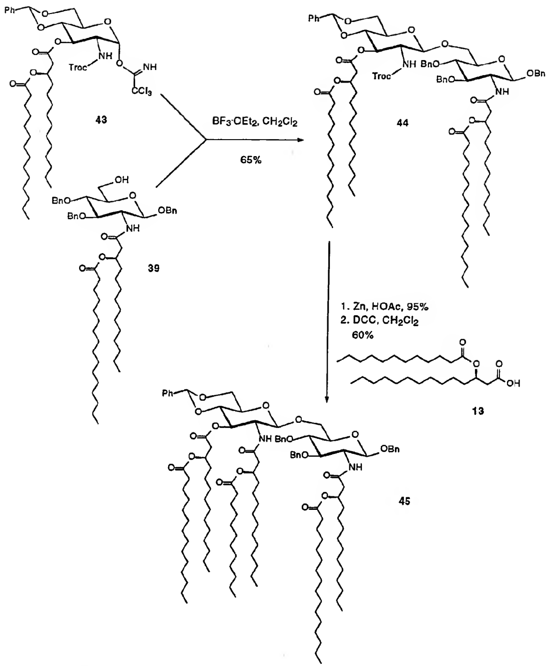


FIG. 12 Synthesis of disaccharide **45**

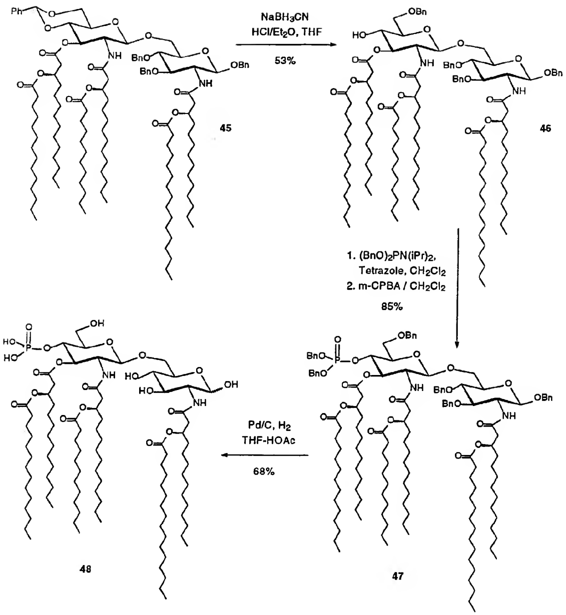


FIG. 13 Synthesis of compound **48**

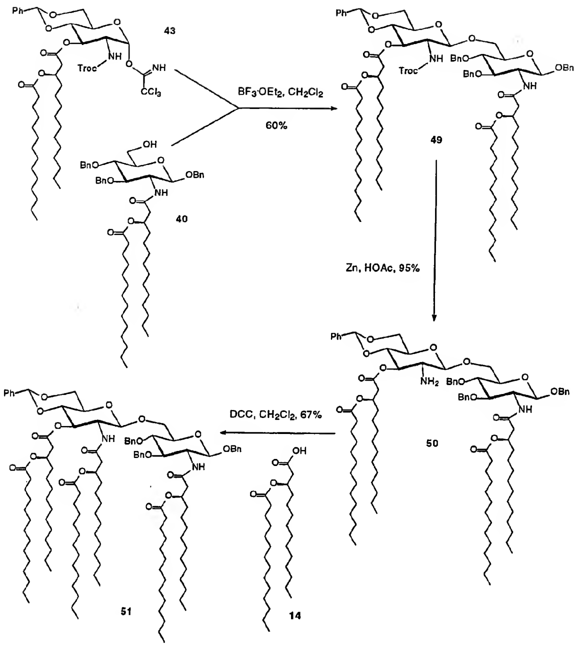


FIG. 14 Synthesis of disaccharide 51

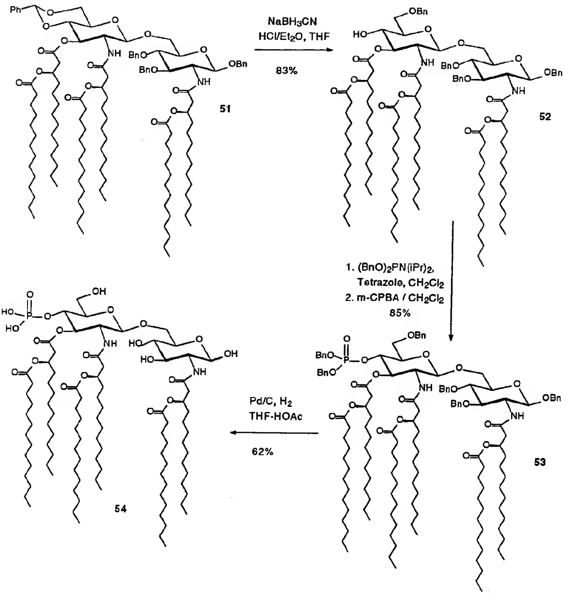


FIG. 15 Synthesis of compound **54**

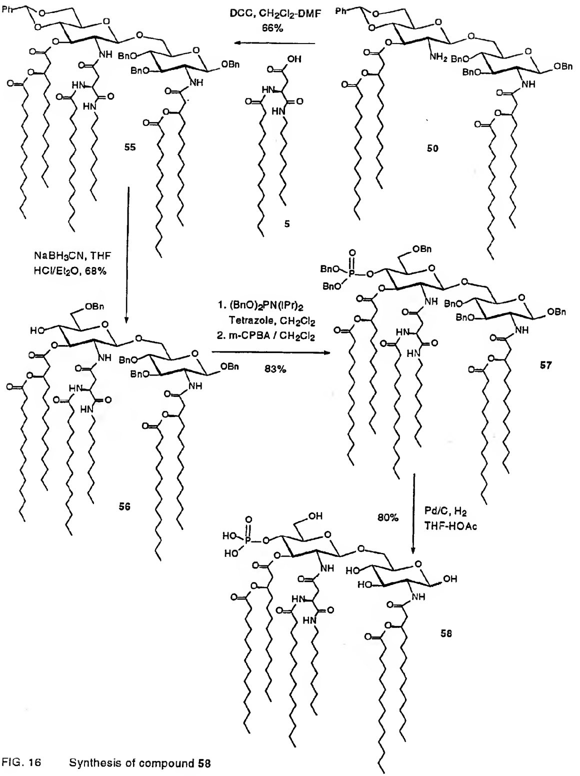


FIG. 16 Synthesis of compound **58**

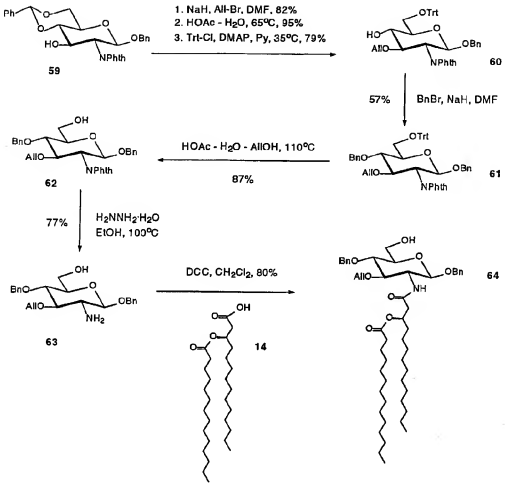


FIG. 17 Synthesis of glycosylation acceptor **64**

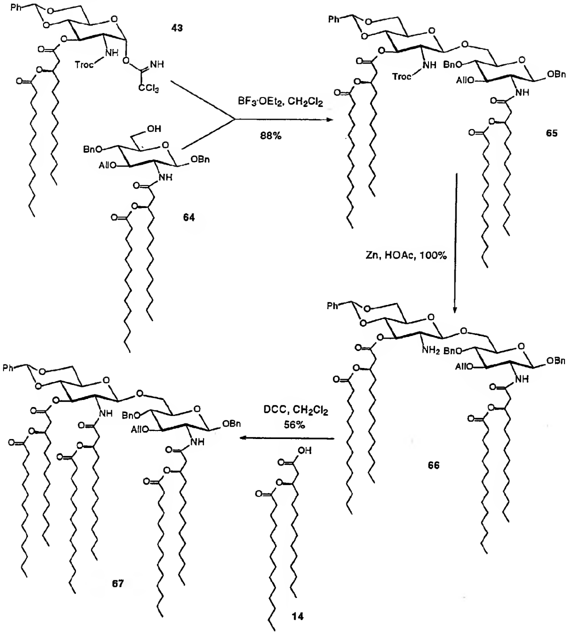


FIG. 18 Synthesis of disaccharide **67**

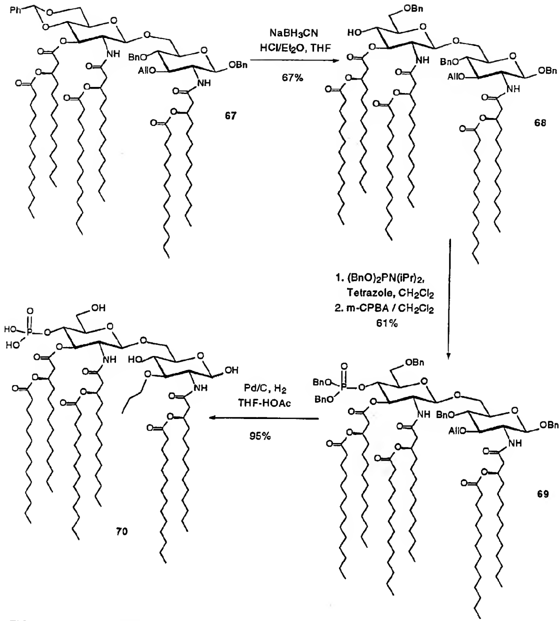


FIG. 19 Synthesis of compound **70**

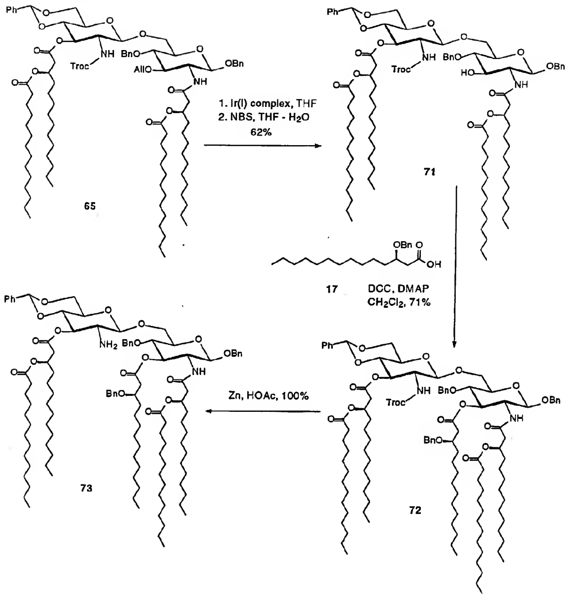


FIG. 20 Synthesis of amine **73**

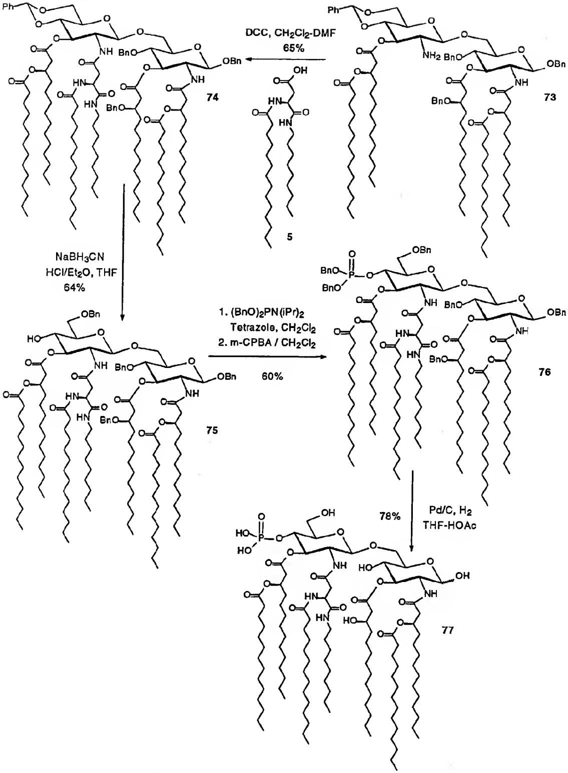


FIG. 21 Synthesis of compound 77

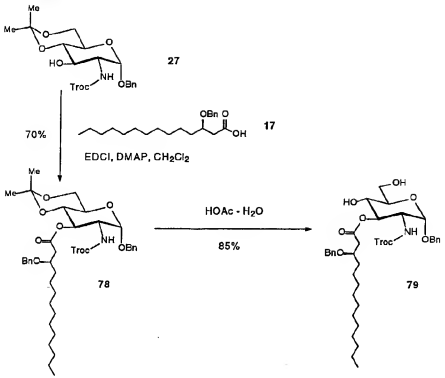


FIG. 22 Synthesis of glycosylation acceptor 79

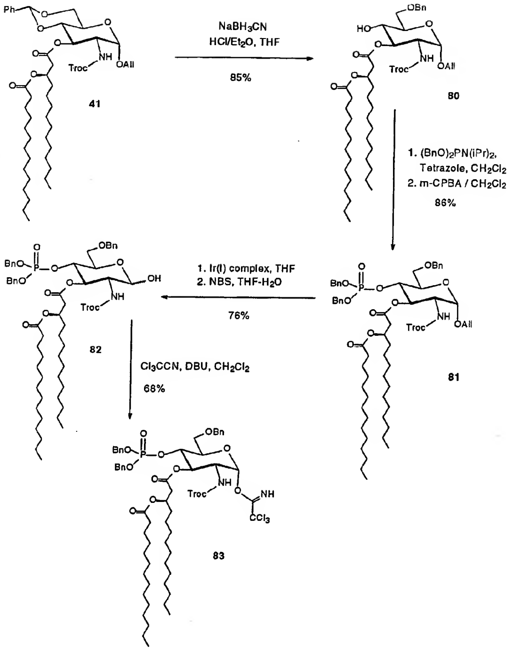


FIG. 23 Synthesis of glycosylation donor **83**

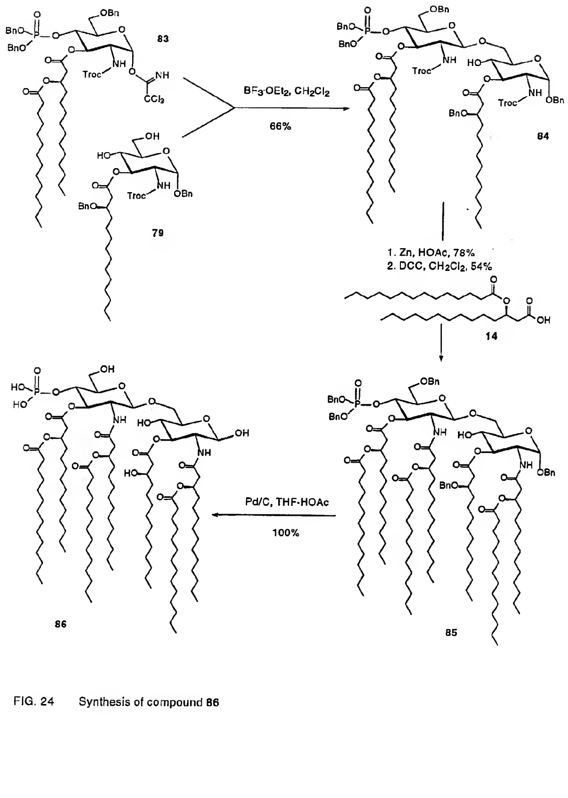


FIG. 24 Synthesis of compound 86

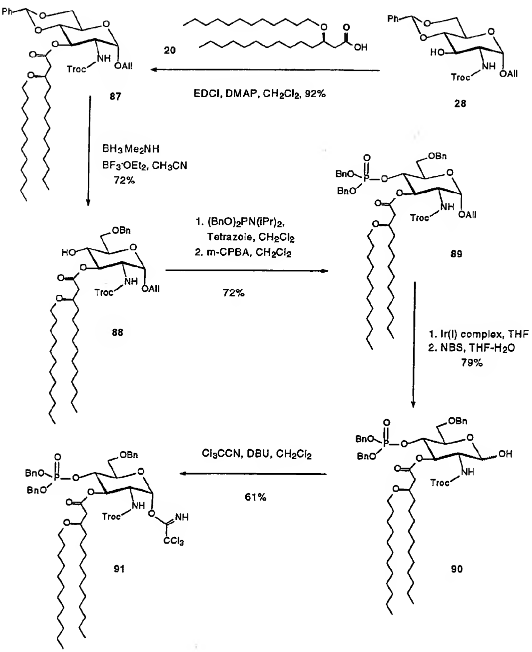


FIG. 25 Synthesis of glycosylation donor 91

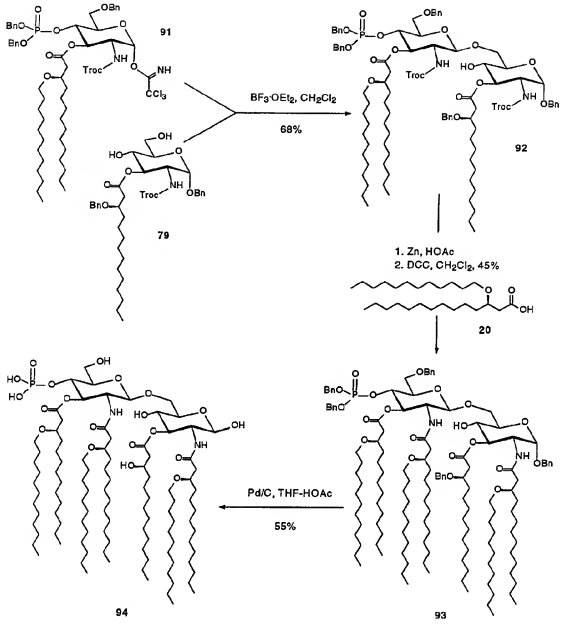


FIG. 26 Synthesis of compound 94

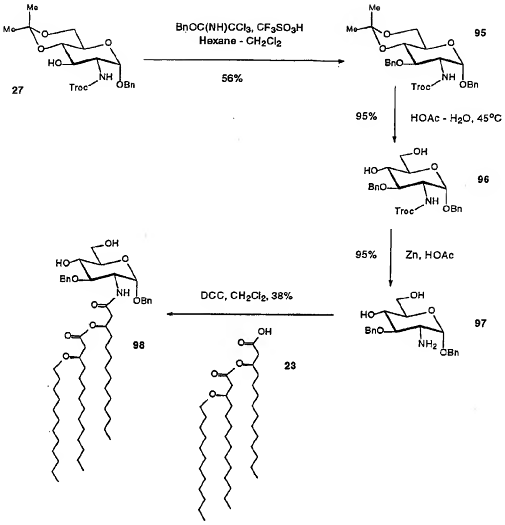


FIG. 27 Synthesis of glycosylation acceptor **98**

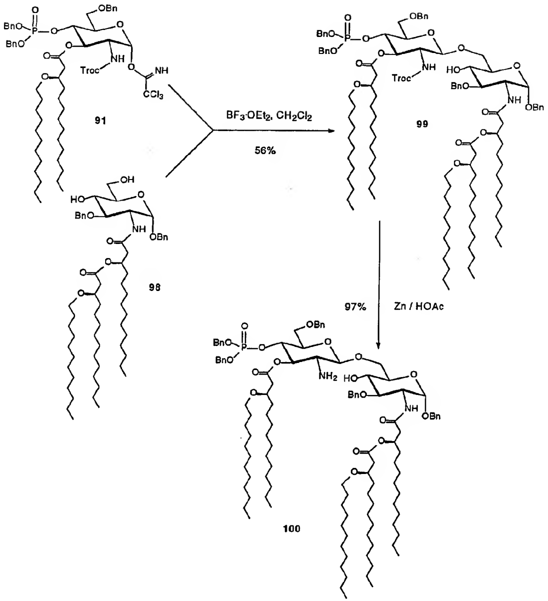


FIG. 28 Synthesis of amine 100

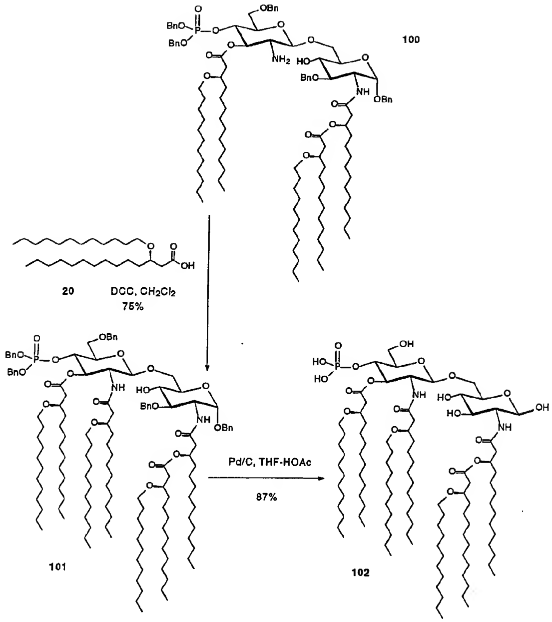


FIG. 29 Synthesis of compound **102**

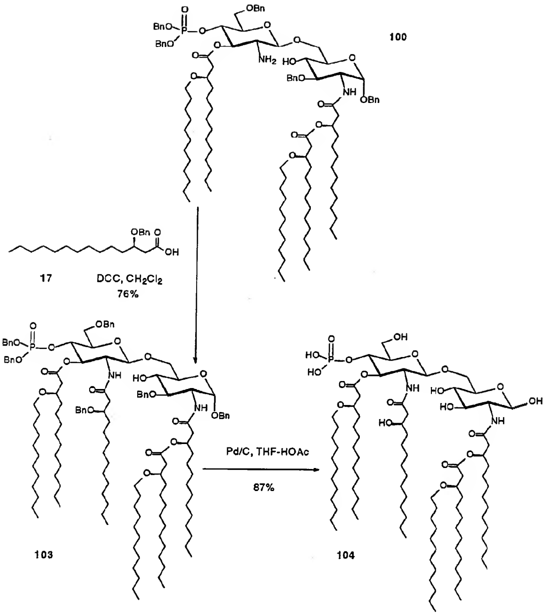


FIG. 30 Synthesis of compound **104**

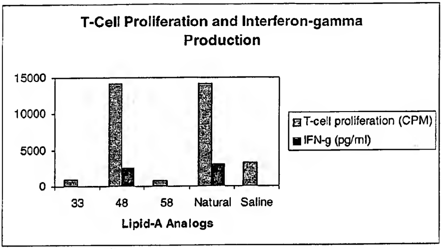


FIG. 31

T-cell proliferation (CPM, counts per minute) and Interferon-gamma (IFN-γ, pg/ml) production in mice immunized with liposomal vaccine which contains MUC1-based synthetic lipopeptide BP1-148 as antigen, adjuvanted with Lipid-A analogs 33, 48, 58 or Natural Lipid-A

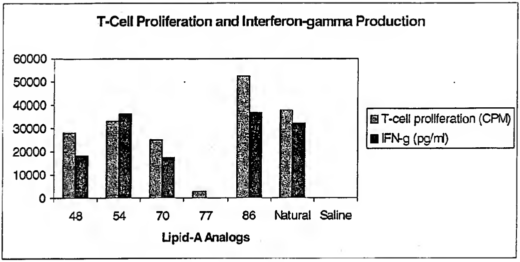


FIG. 32

T-cell proliferation (CPM, counts per minute) and Interferon-gamma (IFN-γ, pg/ml) production in mice immunized with liposomal vaccine which contains MUC1-based synthetic lipopeptide BP1-148 as antigen, adjuvanted with Lipid-A analogs **48**, **54**, **70**, **77**, **86** or **Natural Lipid-A**

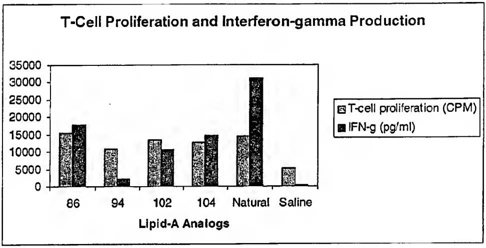
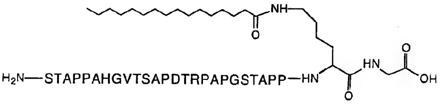


FIG. 33

T-cell proliferation (CPM, counts per minute) and Interferon-gamma (IFN-γ, pg/ml) production in mice immunized with liposomal vaccine which contains MUC1-based synthetic lipopeptide BP1-148 as antigen, adjuvanted with Lipid-A analogs **86**, **94**, **102**, **104** or **Natural Lipid-A**

H_2N-STAPPAHGVTSAPPDTRPAPGSTAPPK(Pal)G-OH



BP1-148

FIG. 34 Structure of lipopeptide **BP1-148**, a modified 25-amino-acid sequence derived from tumor-associated MUC1 mucin (single letter codes of amino acids)

BP1-231 / Malaria NSP-1 (20-39): H_2N-VTHESYQELVKKLEALEDAVK(Pal)G-OH

BP1-232 / Malaria LS1.2 1742-1760: H_2N-HTLETVNNISDVNDFQISKYK(Pal)G-OH

BP1-233 / HepA VP1 (75-92): H_2N-GESRHTSDHMSIYKFMGRK(Pal)G-OH

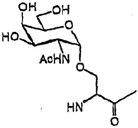
BP1-235 / HBc Ag CTL epitope: H_2N-STLPETTVVRRK(Pal)G-OH

BP1-230 / TB 38 KDa antigen (350-369): H_2N-DQVHFQPLPPAVVKLSDALIK(Pal)G-OH

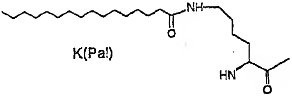
BP1-219: H_2N-GVTSAPDT(Tn)RPAPGSTAS(Lipo)S(Lipo)L-OH

BP1-217: H_2N-GVTSAPDTRPAPGSTAS(Lipo)S(Lipo)L-OH

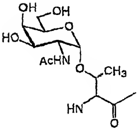
BP1-223: H_2N-GVT(Tn)S(Tn)APDTRPAPGS(Tn)T(Tn)AS(Lipo)S(Lipo)L-OH



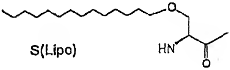
S(Tn)



K(Pal)



T(Tn)



S(Lipo)

FIG. 35 Some examples of peptides and glycopeptides derived from viral antigens and MUC1 protein that can be incorporated into liposome formulations